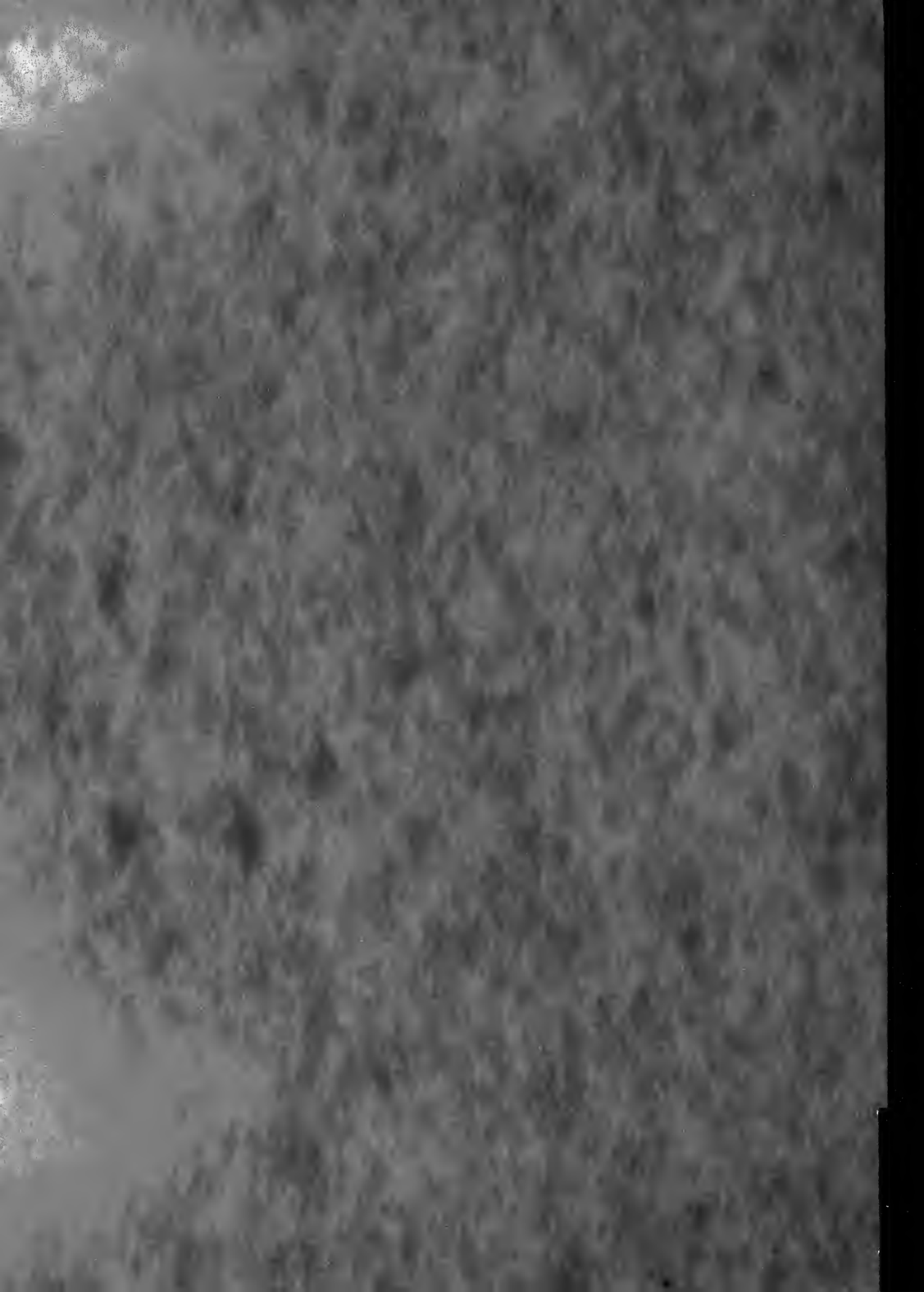


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1992

Center for Biologics Evaluation and
Research
Division of Transfusion Science

Annual report
1991-1992



Center for Biologics Evaluation and Research (CBER)
CD

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DIVISION OF TRANSFUSION SCIENCE

October 1, 1991 through September 30, 1992

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FY92 Annual Report**Report of The Director
Division of Transfusion Science
/ Center for Biologics Evaluation and Research
00 Food and Drug Administration****Introduction**

The FY92 activities and accomplishments of the Division of Transfusion Science (DTS) are described in detail in the following reports of the Laboratory of Blood Bank Practices (LBBP), the Laboratory of Retrovirology (LR), and the Laboratory of Hepatitis (LH). There have been no major changes in the preceding year in the structure or function of the division, but it should be noted that we are completing our fourth year of operating with an acting director for LBBP and the second year with an acting director and deputy for the division. Adequate staffing to permit appropriate response to the large number of blood safety issues that this division deals with routinely continues to be a major concern.

The DTS has always borne a disproportionably large share of the regulatory review activity, and the type of issues requiring resolution frequently result in public discussion through Blood Products Advisory Committee Meetings (BPAC). A very significant amount of staff time is used in planning the meetings, preparing background materials for BPAC members, gathering data (including performing large surveys when necessary), organizing presentations, writing summaries of outcomes and translating outcomes to public policy announcements such as memoranda to all blood establishments.

Significant issues brought to three BPAC Committee Meetings in FY92 included: immune globulin safety; multi-antigen HCV tests and donor suitability determination; false positive EIA test results and the potential relation to recent immunization with influenza vaccine; revised donor screening procedures in relation to confidential unit exclusion, signs and symptoms of HIV and deferral periods for various risk exposures; use of fresh, (untested or pre-tested) blood products; invalidation criteria for EIA testing; and bacterial contamination of platelets (See Appendix 1).

A large part of DTS activity involves the development of regulatory policy guidance documents and interaction with outside groups (See Appendices 2 and 3).

In FY92 DTS completed seven memoranda related to donor screening issues and four in-vitro diagnostic reagent evaluation documents. An additional eight memoranda are in process and five are nearing completion of the review and approval procedure preliminary to signature by the Center Director.

A recent summary of regulatory issues and future needs was prepared by the acting, deputy director (See Appendix 4). Although the reorganization will change to some degree the responsibility for addressing these issues, the summary accurately reflects the areas which will require resources.

Change in Physical Location

The move of the director's office, LBBP, LH and parts of LR staff to Nicholson Lane Research Center was finally achieved November 16, 1991. Unfortunately, uncorrected facility problems, though identified well in advance, have prevented the completion of LR's move. It has taken considerable staff effort to achieve the DTS relocation, but the improvement in physical environment has been much appreciated by all staff. Difficulty with transportation to meetings and lack of parking at NIH have been the only serious negative impact of the move.

Contract with American Institutes of Research (AIR)

FDA Contract #223-91-1002

This 2 year contract titled "Increasing the Safety of the Blood Supply by Screening Donors more Effectively", was awarded April 20, 1991. This study is a follow up of a contract study with AIR concluded in 1990 whose purpose was to develop new donor AIDS screening materials and to experimentally evaluate them on-site in blood establishments. The goals of the current study are to develop a new process for donor screening that addresses the distinct needs of first time donors, repeat donors and training for blood center staff.

This fiscal year, AIR has completed its analysis and development phases. They have developed and are in the process of final testing a new screening process based on an interactive computer based prototype. FDA has received four sets of materials for review that will be tested in two field sites during the final phase of the project. These are a computer assisted donor interview station, a donor screener station, a training program for donor screeners and new pre/post-donation cards. These materials include a more effective informed consent process and two distinct donation tracks that distinguish between first-time and repeat donors. The findings from this study will provide FDA with important information concerning the feasibility of utilizing a new process for donor screening and a training program for health historians. Field testing is scheduled for September with the evaluation phase to be completed in April of 1993.



Increasing the Safety of the Blood Supply by Screening Donors More Effectively

On March 1, 1991, the American Institutes for Research (AIR) began a study for the Food and Drug Administration (FDA) aimed at increasing the safety of the blood supply by improving the process of donor screening. The goal of this study is to develop a new process for donor screening that addresses the distinct needs of first-time donors, repeat donors, and blood center staff. The new process will include two main components:

- a streamlined and simplified set of procedures for first-time and repeat donors to follow during the process of donating blood. These procedures may include separate tracks for repeat and first-time donors.
- a training program for blood center staff to ensure that they are thoroughly familiar with all deferral criteria, screening information, and screening procedures.

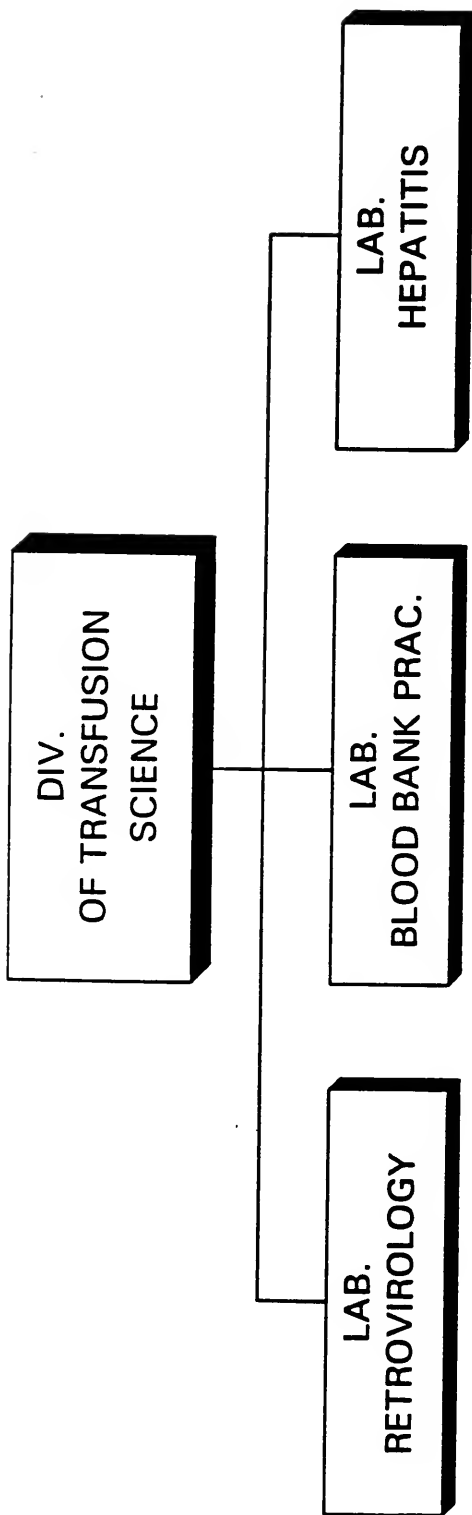
The study is divided into three phases: an analysis phase, a development phase, and an evaluation phase. During the analysis phase, currently in progress, AIR is analyzing the feasibility of alternative strategies for modifying current donor procedures and training programs for blood center staff. As part of the analysis phase, AIR will be reviewing relevant literature, assessing technological alternatives, and interviewing current and former donors to identify the strengths and weaknesses of current donor procedures. AIR will also be interviewing medical directors, supervisors, health historians, and phlebotomists at several blood centers to determine the requirements of staff involved in blood collection.

During the development phase of the study, AIR will develop a prototype for a new donation procedure and a new training program. The prototypes will include a new, integrated package of communication materials for donors and a set of training materials and proficiency tests for blood center staff. During the development phase, AIR will formatively test all of these materials in a controlled setting to ensure that they meet acceptable standards of comprehensibility and usability.

Finally, during the evaluation phase, AIR will test the effectiveness of the prototypes at actual blood donation sites. This testing will include both fixed and mobile sites. Within the fixed sites, the testing will include both whole blood donors and voluntary, non-remunerated pheresis donors.

At the end of the evaluation phase, AIR will prepare a detailed report describing the results of the testing. The study will be completed in March, 1993.

WASHINGTON RESEARCH CENTER
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WASHINGTON, DC 20007
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August 1992

DIVISION OF TRANSFUSION SCIENCE

Acting Director
P. Ann Hoppe

Acting Deputy Director
Jay Epstein, M.D.

Assistant to the Director
Linda Smallwood, Ph.D.
Martha Wells, M.P.H.

Staff

Stiney
Bishop
Houk
McMillian
McCullough

Retrovirology

Chief. Epstein

Reg.Coord. P.Mied

Blay
Buck
Carrow
Francis
Geyer
Joshi
Heredia
Hewlett
Lee
Malarky
Mayner
Neiger
Panneerselvam
Poffenberger
Riordan
Roberts
Ruta
Sausville
Shawver
Uday

Hepatitis

Biswas

Wilson

Claggett
Gilbert
Mitchell
Nedjar
Saum
Zhai

Blood Bank
Practices

Hoppe (Acting)

Santos

Callaghan
Capen
Denham
Frantz-Bohn
Hall
Holness
Jones
Kochman
Morrow
Northern
Wellstood

**DIVISION OF TRANSFUSION SCIENCE
NUMBER OF EMPLOYEES**

FISCAL YEAR	OD	LBBP	LH	LRV	TOTAL
1990	8	8	8	19	43
1991	6	7	7	20	40
1992	8	12	8	22	50

PUBLICATIONS

1. Blay R, Hernandez D, Betts M, Clerici M, Lucey DR, Hendrix C, Hoffman T, Golding B. *Brucella abortus* stimulates human T cells from uninfected and HIV-infected individuals to secrete IFN γ : Implications for use of *Brucella abortus* as a carrier in the development of human vaccines, *AIDS Res Human Retroviruses* 1992;8(4):479-486.
2. Castro A, Pedreira P, Soriano V, Hewlett I, Joshi B, Epstein J, Gonzales-Lahoz. Kaposi's Sarcoma and disseminated tuberculosis in an HIV negative individual, *Lancet*, 1992: p868
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5. Hoppe, P.A., Progress in Achieving International Consensus on Reagent Standards, *Transfusion Today*, 1991; 12:12-13.
6. Hoppe, P.A., Substantially Revised Quality Assurance Chapter for AABB Technical Manual 11th Ed., In press.
7. Hoppe, P.A., Biosafety, **AABB TECHNICAL MANUAL**, 11TH Ed., American Association of Blood Banks, Arlington, VA, In press.
8. Hoppe, P.A., Records, **AABB TECHNICAL MANUAL**, 11TH Ed., American Association of Blood Banks, Arlington, VA, In press.
9. Idowu AD, Fraser-Smith EB, Poffenberger KL, and Herman RC. Deletion of the herpes simplex virus type 1 ribonucleotide reductase gene alters virulence and latency in vivo, *Antiviral Res* 1992; 17:145-156
10. Joshi B, Epstein J, Riordan G, Pollock L, and Hewlett IK. Benzo alpha pyrene induces HIV-1 expression in chronically infected promonocytic cells. In: *Vaccines*. Cold Spring Harbor Laboratory, 1992; 247-252.
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12. Nedjar S, Mitchell F, Biswas R. Enzymatic co-amplification and simultaneous detection of hepatitis B virus and hepatitis C virus-specific genomic sequences in donor sera. *Transfusion*, vol. 31(1991) 58s.

13. Neurath AR, Haberfield B, Joshi B, Hewlett IK, Strick N, and Jiang S. Rapid prescreening for antiviral agents based on their inhibitory activity in site directed immunoassays. I. The V3 loop of gp120 as target, *Antiviral Chemistry and Chemotherapy*. 1991; 2(5): 303-312.
14. Poffenberger KL, Raichlen P, and Herman RC. In vitro characterization of a herpes simplex virus type 1 ICP2 null mutant, *Virus Genes*, 1992 (in press).
15. Prince AM, Reesnick H, Pascual D, Horowitz B, Hewlett IK, Murthy KK, Titlie CM, and Eichberg J. Prevention of HIV infection by passive immunization with HIV immune globulin, *AIDS Res. Hum. Retro.* 1991; 7(12): 971-973.
16. Sawyer LA, Carrow E, Snoy P, Hewlett IK, Quinnan GV and Anand R. Variable infections with and serologic responses of rabbits to five different HIV-1 strains, including one neural tissue isolate, *Microbios.* 1992 (in press).
17. Selvam MP, Mayner RE, Buck SM, Epstein JS. A novel sensitive radioimmunoprecipitation assay for the detection of antibodies to human immunodeficiency virus type 2 (HIV-2), *Biochem Biophys Res Commun* 1992 (in press).
18. Soriano V, Hewlett I, Friedman-Kien A, Huang Y, Tor J, Epstein J. Definitive exclusion of HIV infection in a Kaposi's Sarcoma (KS) bisexual man: suggestions of a pathogenic model for KS, *Vox Sanguinis* 1992 (in press).
19. Soriano V, Hewlett IK, Tor J, Esteve M, Granada I, Muga R, Epstein J, and Nedrano L. Evidencia de infeccion por el virus de la inmunodeficiencia humana en sujetos de colectivos de alto riesgo que son seonegativos, *Med. Clinica.* 1991; 97: 441-445.
20. Udaykumar, Sarin R, and Saxena RK. Analysis of circulating immune complexes in tuberculosis: Levels of specific antibody and antigen in CIC and relationship with serum antibody, *Clinical Digest Series*, 1992 (in press).
21. Udaykumar and Saxena RK. Acid pH-induced changes in the immunoreactivity of specific antigen and antibody in circulating immune complexes from tuberculosis sera, *J. Clin. Lab. Anal.*, 1992; 6:194-200.
22. Wormser G, Bitker S, Forseter D, Hewlett IK, Argani I, Joshi B, Epstein J, and Bucher D. Absence of infectious human immunodeficiency virus type 1 in natural eccrine sweat, *J. Inf. Dis.*, 1992; 165: 155-158.

FY92 Annual Report

Laboratory of Blood Bank Practices

This small staff has in FY92 considerably improved the handling of the regulatory review process for their area of responsibility. Although several staff were relatively new to the FDA, virtually all have participated in the development of written SOP's for all parts of the review process (See Appendix 5). This work has proceeded smoothly while staff have concurrently continued to reduce the backlog of regulatory review from over 1000 open files at the start of FY92 approximately 250 at the close of year. All staff have worked cooperatively to continuously improve the quality and efficiency of the review work. Examples of projects and innovation to provide clear guidance on FDA requirements and the review process include the following:

1. Developed a notice to be included with all outgoing correspondence concerning pending applications potentially affected by changes related to FDA's four memoranda of 23 April 1992.
2. Notified large Source Plasma manufacturers concerning any submission pending longer than 6 months without a CBER response, and requested attention to withdrawing obsolete procedures.
3. Wherever possible, uniform language was developed and all "form" letters/standard language paragraphs were reviewed and updated; after clearance by DPC, the approved items were put on every word processor so that one stroke produces correct wording for all common situations.
4. Historical policy files were upgraded by appointing a key "policy keeper" for each review area.
5. Information was collected from all Source Plasma centers accepting donors with elevated ALT's, and a process was initiated to reach a policy decision.
6. Developed a new policy on FDA-cleared equipment that permits early use by licensed blood establishments, while assuring consistent documentation review during inspections.
7. Initiated a task force to develop comprehensive review guidance for EIA test equipment.
8. Completed draft of "Computer Users Guidelines" and a memo on "Retrospective Validation" of Blood Bank Computer Systems."
9. Coordinated with DPC a system to assign tracking numbers to all incoming correspondence, and met with DPC to reduce confusion on handling correspondence.
10. Established a task force on leukocyte reduction to review filter specifications and performance, and establish revised licensing criteria for leukocyte-reduced products.
11. Targeted all American Red Cross transformation issues for handling by a small group of key staff to ensure that no delays occur in review of revised procedures.
12. Provided in-depth assessment of a software program for

blood bank use and evaluated the attendant hazards.

13. Drafted a revised Source Plasma Application in a "check list" format that will save both manufacturers and FDA enormous resources in preparation and review of applications.

14. Participated in three compliance actions through the evaluation of official samples from three different cases. In each case, samples were submitted by the District Office to the sample custodian, DPQC, and referred to LBBP staff for evaluation. Evaluation included determination of sample integrity, observation of sample appearance, performance of laboratory testing to determine presence or absence of various factors, including antibodies, and preparation of reports of results. Reports were then submitted to the Office of Compliance for their use in determining follow-up actions.

15. The increased number of automated plasma collection devices with varying capacities for tailoring each collection to the specific donor has resulted in the existence of multiple Food and Drug Administration approved nomograms which specify, for each piece of equipment, the maximum volume of plasma to be harvested from each donor category. Because multiple equipment types commonly coexist in a location, the potential for error due to application of an inappropriate nomogram is significantly increased. Some Source Plasma manufacturers have requested and received approval for simplified nomograms. The FDA supports this type of process change which potentially improves the consistency of procedures for manufacturing and minimizes the opportunity for staff error. The Center for Biologics Evaluation and Research has issued a notice of policy concerning rapid implementation of a simplified volume nomogram. The use of this simplified nomogram does not require advance approval of amendments to licenses. This will decrease the review work that has to be submitted to CBER and save both industry and FDA resources.

Quality Assurance Workshop and Guidelines

A central focus of activity in FY92 has been the development of Quality Assurance (QA) guidance for blood establishments. This effort has a very high priority in FDA and a high level of interest with outside groups. The workshop on January 21 and 22 attracted over 500 participants and the QA task force has dealt with a large number of comments on the proposed guidance document (Appendix 6).

As manufacturing processes for blood products become more complex, the potential for errors and accidents directly related to these new processes increases. FDA believes that an urgent need exists to implement QA programs in blood establishments to decrease the number of errors and accidents, and to increase the safety of the nation's blood supply.

Speakers from industry presented key components of successful QA programs, current QA initiatives within private industry, internal QA audit programs, donor deferral registries, personnel training and competency evaluation. FDA speakers discussed the role of QA/OC (quality control) units equipment validation and computer systems requirements, and outside testing services.

Staff Training Initiative

In addition to extensive work on the standard operating procedures for the Laboratory of Blood Bank Practices, weekly seminars have been held in combination with staff meetings. These sessions have been used to give the staff an opportunity to practice lectures they will give outside and to provide organized information on special topics or current issues generating regulatory questions.

Topics which have been presented are irradiation of blood and blood products, quality assurance in the blood bank, update on the FDA quality assurance initiative, bacterial contamination of platelets, update on liaison meetings with blood bank community, update on computer software regulatory problems, fresh blood issue, Freedom of Information Act, use of the electronic mail system, and medical devices review. Topics which are in the process of being scheduled for fall will include short supply agreements, leukocyte reduction of blood and blood products, new in-vitro products, new test for anti-HIV, test invalidation, CLIA, von Willebrand's disease, and processing of bone marrow for transplantation.

Publications

1. Hoppe, P.A., Interim Measures for Detection of Bacterially Contaminated Red Cell Components, *Transfusion*, 1992; 199-201.
2. Hoppe, P.A., Progress in Achieving International Consensus on Reagent Standards, *Transfusion Today*, 1991; 12:12-13.
3. Hoppe, P.A., Substantially Revised the Quality Assurance Chapter for AABB Technical Manual 11th Ed., In press.
4. Hoppe, P.A., Biosafety, ABB TECHNICAL MANUAL, 11th Ed., American Association of Blood Banks, Arlington, VA, In press.
5. Hoppe, P.A., Records, ABB TECHNICAL MANUAL, 11th Ed., American Association of Blood Banks, Arlington, VA, In press.

LBBP License Review ActivitiesSummary Report of License Submissions Reviewed
08/01/91 - 07/27/92

	Establishment License	Product License	INDs
Whole Blood - includes In-Vitro products	21	130	
Source Plasma	55	156	
INDs (Whole Blood, Source Plasma, or multi-product)			13

OTHER ACTIVITIES (includes SOP review)

Whole Blood Correspondence includes In-Vitro products	237
Source Plasma Correspondence	236
510(k)s, PMAs, NDAs	83
Fax inquiries	305
Precedent setting Health Hazard Evaluations	32
9 - involving infectious disease contamination issues or testing problems 13 - related to incorrect establishment processing of blood products 10 - related to a defective product involved with blood processing	

There was a flow chart originated to be used in handling the recall of products potentially contaminated with bacteria. This flow chart lists criteria to be used in classifying bacterially contaminated products.

This staff also reviewed Inspection Reports of blood banks and plasma centers for policy conformance and provided health hazard evaluations on many potential suspension and revocation actions. They also served as consultants on many occasions for the field inspectors via phone calls and faxed inquiries. These inquiries required immediate response during the course of the field inspection and, therefore, disrupted routine assignments significantly.

Facility Inspections

In-Vitro	10
Whole Blood and Source Plasma	12

SUMMARY OF LOT RELEASE ACTIVITY

	1989	1990	1991	1992 TD	1992 PROJ
AHG	94	91	102	99	119
BGR	496	475	521	376	451
ABO	109	117	144	87	104
D	92	66	69	54	65
Rh-Hr	127	118	113	87	104
Other	167	171	195	148	178
BG Subs.	1	3	0	4	4
TOTALS	590	566	623	479	575

NOTE: An overall decrease in the number of products for lot release can be attributed to an increase in the number of manufacturers being granted exemption from lot release for Anti-A, Anti-B, Anti-A,B, Anti-D, and Anti-Human Globulin reagents.

DETAIL REPORT OF REAGENT LOT RELEASE 1992, 10/1/91 to 7/27/92

	ABO	D	Rh-Hr	Other	AHG	BG Subs	TOTALS
Manufact.No.1	0	0	0	4	0	0	4
Manufact.No.2	0	0	0	0	0	0	0
Manufact.No.3	3	8	8	10	13	0	42
Mnaufact.No.4	0	2	0	0	4	0	6
Manufact.No.5	6	0	0	0	0	0	6
Manufact.No.6	15	0	0	0	0	0	15
Manufact.No.7	15	10	15	29	30	0	99
Manufact.No.8	7	3	8	15	0	0	33
Manufact.No.9	13	3	8	21	11	0	56
Manufact.No.10	0	15	13	28	17	0	73
Manufact.No.11	28	13	35	41	24	4	145
TOTALS	87	54	87	148	99	4	479

Reagent Red Blood Cell samples from new donors 10/1/91 to 7/27/92

830 samples received for evaluation and review; many examples droplet frozen and entered into the rare cell inventory.

Outside Lectures

October 1991 National Heart Lung and Blood
Institute/Bethesda - AIDS Ad Hoc (Epstein
substituted)

November 1991 AABB/Baltimore - 4 events: Inspection
Workshop lecture, RAP session on donor
selection, chair, Ask the FDA Inspector, Ask
the Experts (Hoppe)

 National Blood Resources Education
Program/Bethesda (Hoppe)

 American Red Cross/St. Paul, MD - 1/2 day
Workshop for Technical Manager Training
Course (Hoppe)

December 1991 World Health Organization/Geneva - Develop
Standards for Collection, Processing and
Quality Control of Blood, Blood Components
and Plasma Derivatives (Hoppe)

January 1992 FDA/HCFB/Bethesda, MD - General Principles of
Quality Assurance (Hoppe/Frantz-Bohn et al)

 American Association of Blood
Banks/Arlington, VA - Immunohematology Update
(Hoppe)

February 1992 Council of Community Blood
Centers/Scottsdale, AZ - Contemporary
Operational Issues/Blood Quality Assurance
(Hoppe)

March 1992 Council of Europe/Lund, Sweden - US Practices
for Confidential Unit Exclusion (Hoppe)

 FDA Orientation for Small Business
Representative/Bethesda, MD - Overview of
Division of Transfusion Science (Wells)

April 1992 South Central Association of Blood
Banks/American Association of Blood
Banks/Fort Worth, TX - Working Together
Towards Compliance (Hoppe)

 Center for Disease Control/Atlanta, GA -
Donor Blood Studies Consultation (Hoppe)

 Pennsylvania Association of Blood
Banks/Atlantic City, NJ - Serious Adverse
Reactions and Disaster in the Blood Bank
(Hoppe - 2 lectures)

SAFMILS Armed Forces Blood Program/San Antonio, TX - FDA Compliance Workshop (Frantz-Bohn)

CHC User's Group/Houston, TX - Computers and inspections/based on Section K of the 483 (Capen)

May 1992

Specialists in Blood Banking
Students/Kensington, MD - Overview of CBER (Hoppe), Quality Assurance (Frantz-Bohn), Source Plasma (Callaghan), FDA Regulation of IVD Blood Bank Reagents (Kochman), SMDA and How It Affects the Blood Bank (Northern), Whole Blood (Denham)

Council of Europe/Luxembourg - U.S. Practices for Confidential Unit Exclusion (Hoppe)

North Atlantic Treaty Organization/Brussels, Belgium - Civil/Military Blood Coordination (Cancelled due to Reorganization)

Tennessee Association of Blood Banks/Knoxville, TN - FDA Update (Frantz-Bohn)

June 1992

World Health Organization/Geneva - Global Blood Safety Initiative (Hoppe)

Scotblood/Stirling, Scotland (Cancelled due to Reorganization)

International Society of Blood Transfusion/Montpelier, France - Uniform Labeling (Cancelled due to Reorganization)

September 1992

American Association of Blood Banks/St. Paul, MN - Current Issues (Hoppe)

American Association of Blood Banks/Rhode Island - Current Issues (Hoppe)

Kentucky Association of Blood Banks/Lexington, KY - Quality Assurance Issues (Frantz-Bohn substituted)

LIAISON:

AABB Standards Committee (Hoppe)

AABB Ad Hoc Committee to review Donor Screening Procedures (Hoppe)

AABB Extracorporeal Therapy Committee (Santos)

AABB Transfusion Transmitted Disease Committee (Epstein)

AABB Transfusion Practices Committee (Holness)

AABB Safety Committee (Hoppe)

AABB Technical Manual Committee (Hoppe)

AABB Autologous Transfusion Committee (Denham/Hoppe)

AABB Information Systems Committee (Hoppe/Northern)

AABB Scientific Program (Hoppe/Epstein)

AABB Technical Quality Assurance (Frantz-Bohn)

EPA Medical Waste Policy Committee (Hoppe)

Council of Europe - Committee of Experts and subcommittee on Automation and Quality Standards (Hoppe)

WHO Donor Standards Committee (Hoppe)

WHO Reagent Production Committee (Hoppe/Kochman)

ISBT Enzymes Standardization Committee (Hoppe/Kochman)

ISBT Antiglobulin Standards Committee (Hoppe/Kochman)

ISBT Monoclonal Reagents Standards Committee and Workshops (Hoppe)

NHLBI Transfusion Safety Study Policy Board (Hoppe)

NHLBI AIDS Ad Hoc Committee (Hoppe/Epstein)

National Blood Resources Education Program Policy Board (Hoppe)

A2LA (American Association of Laboratory Accreditation)-FDA Liaison to the Board of Directors (Hoppe)

Health Care Financing Administration Liaison (Hoppe)

NBREP Subcommittee on Autologous Transfusion (Hoppe)

ANNUAL REPORT**LABORATORY OF HEPATITIS****OCTOBER 1, 1991 TO SEPTEMBER 30, 1992**

Personnel changes have again occurred in the Laboratory. Since October 1, 1991, a Staff Fellow and a Visiting Scientist have joined the Laboratory and a Staff Fellow has left the laboratory. These personnel changes have impeded research projects and have hindered product reviews. Reviews are handled by one person full time and 3 people at about 20% of their time.

Significant activities and accomplishments during the period covered by this report include the following:

RESEARCH

1. The safety of immune globulin products, in regard to hepatitis C (HC), manufactured from anti-HCV depleted plasma pools, is being studied in chimpanzees and is still in progress. As reported last year, plasma from anti-HCV negative Source Plasma donations was pooled and fractionated into intravenous immune globulin (IVIG). Samples from the unprocessed plasma pool were inoculated into 2 chimpanzees. These animals became infected with HCV as demonstrated by the development of anti-HCV, ALT elevations and the detection of HCV-RNA in the plasma. IVIG was then inoculated into 3 chimpanzees. If any of these animals had developed HC, it would have implied that screening for anti-HCV is deleterious to the safety of IVIG. If none of these animals had developed HC, it would suggest that screening plasma for anti-HCV will not adversely affect immune globulin safety. Twelve months after inoculation of IVIG had passed, none of the animals showed any evidence of hepatitis infection. This data was used as the basis to recommend the screening of source plasma for anti-HCV. Subsequently two animals were challenged with known infectious human plasma to ensure the capability of infectivity, and a third animal remained unchallenged and serves as a control. One of the animals that was challenged with HCV has seroconverted.
- 2.. The sequence of appearance of HCV-RNA and of the different antibodies to hepatitis C virus (HCV) epitopes following experimental infection of two chimpanzees with HCV, have been investigated. The chimps were infused with unprocessed, pooled plasma from donations that were non-reactive for anti-HCV. Serum samples were obtained prior to infusion, and then weekly subsequent to infusion. These samples were tested for anti-HCV, HBsAg, anti-HBs, anti-HBc and alanine aminotransferase (ALT). Serum samples were also tested for the presence of HCV-RNA by polymerase chain

reaction assay (PCR) and for antibodies to c100-3, C22-3, c33c and 5-1-1 by the immunoblot, RIBA II test. The two chimpanzees showed different patterns in the sequential and temporal appearance of HCV-RNA and of antibodies to the different HCV epitopes. HCV-specific genomic sequences were detected in serum within one week after infusion in one animal and 9 weeks in the other chimp. Elevations in ALT values were observed 3 weeks subsequent to infusion in both animals.

3. A sensitive and specific two stage polymerase chain reaction (PCR) method was developed for the simultaneous amplification and detection of specific genomic sequences of hepatitis B virus (HBV) and hepatitis C virus (HCV) in donor sera. Initially, HCV-RNA was reverse transcribed to cDNA. This cDNA, and DNA from HBV, were then co-amplified by using primer pairs derived from conserved regions of HBV and HCV nucleotide sequences. The specificity of PCR products was confirmed by liquid hybridization analysis using ³²P-end labeled oligomer probes specific for HBV and HCV nucleotide sequences. Independent sets of sera, positive and negative by PCR for either HBV-DNA or HCV-RNA, were used as controls. Nine donor sera, antibody reactive for both HBV (HBsAg and anti-HBc) and HCV (anti-HCV), were tested. Our assay detected HBV and HCV-specific genomic sequences in 9/9 sera reactive for both HBV and HCV antibodies. The ability to co-amplify and detect HBV and HCV sequences, non-isotopically may be particularly useful in the clinical laboratory setting.

REGULATORY

1. Five of six test kits used to detect antibody to hepatitis B core antigen (anti-HBc) have been granted surveillance status.
2. Two improved hepatitis C assays were licensed. These assays incorporated more epitopes of the HCV genome thereby enabling the test kits to detect additional antibodies to HCV antigens. This improvement will result in more infectious units being eliminated from the blood supply. Accordingly, a revised Memorandum to blood establishments recommending the testing of certain blood products for antibody to hepatitis C virus (anti-HCV) using newly licensed multi-antigen test kits was finalized on 23 April 1992. This document also recommended that Source Plasma and Source Leukocytes be tested for anti-HCV (based on LH chimpanzee experiments) as well as other pertinent guidance.
3. A working group was formed consisting of personnel from the Laboratory of Hepatitis, Laboratory of Retrovirology and the Office of Compliance to address invalidation of test results, using either external controls or donor test data,

when using viral marker test kits to screen donor blood. A presentation was made to the Blood Products Advisory Committee on 28 May 1992 and at that time, public comment was also solicited. Efforts are now focused on the development of an establishment memorandum integrating issues discussed at the Blood Products Advisory Meeting and other safety concerns.

4. CDC raised a concern to FDA that certain anti-HBs test kits may be so analytically sensitive that these tests may detect minute quantities of anti-HBs in serum and possibly mislead clinicians to believe sufficient anti-HBs is present for protection against HBV. FDA responded by informing CDC that 1.) anti-HBs test kits are neither designed nor lot released by FDA based on the detection of protective levels of anti-HBs and 2.) and testing all the licensed anti-HBs test kits against the WHO standard (10 mIU/mL is considered to be protective) and reporting that all of the test kits detect much less than 10 mIU/mL (≈ 1 mIU/mL). Based on that information, CDC is currently reviewing the frequency of specimens in the 1-10 mIU/mL region and reassessing whether or not these individuals may be unprotected for HBV.
5. A contract to manufacture CBER hepatitis lot release reference panels was offered and proposals are under review. The program will be a joint effort by the contractor and the Laboratory to manufacture panels for HBsAg, anti-HBs, anti-HBc and anti-HCV. If approved, the two year project is planned to begin in FY92 and is anticipated to provide a sufficient supply of panels for lot release and a standardized framework to manufacture future panels.
6. A number of requests from blood establishments, FDA field investigators and the CBER Office of Compliance concerning interpretations and violations of test methods and CBER recommendation memorandums continue to be processed by Laboratory personnel.
7. During FY92, the Laboratory's main activity centered on hepatitis C: as mentioned in point 2., FDA approved two multi-antigen anti-HCV enzyme immunoassays and four recombinant HCV proteins for further manufacture. In addition, a second 510(k) for external controls for HBsAg assays was processed. With less than a full complement of staff, the laboratory tested 398 lots of reagents either in support of licensure or as a lot release function. Regulatory actions were performed on sixty-eight submissions (originals, supplements, amendments, labeling transmittals) including two 510(k)s, one PMA, 34 INDs, 29 PLAs, 12 ELAs, 2 Blood Establishment SOPs and 7 labeling transmittals. In addition, nine factory inspections were performed.
8. In the course of test kit lot release testing, the Laboratory detected a total of 9 errors related to lot

release protocols (e.g., calculation errors, missing data or uninterpretable information, labeling etc.) by manufacturers. Progress has been made in that this number is a reduction from FY91 (n = 18).

9. On April 23, 1992, the Memorandum, "Exemptions to Permit Persons with a History of Viral Hepatitis Before the Age of Eleven Years to Serve as Donors of Whole Blood and Plasma: Alternative Procedures, 21 CFR 650.120", was issued to all blood establishments.

A brief summary of the contents of this memorandum follows:

Until the regulations, 21 CFR 640.3(c)(1) and 640.63(c)(11), that preclude all persons with a history of hepatitis from donating, are amended, the FDA will consider procedures acceptable that permit a person with a history of viral hepatitis before the age of eleven years to serve as a donor of Whole Blood and Source Plasma.

**FUTURE PLANS
LABORATORY OF HEPATITIS
OCTOBER 1, 1991 TO SEPTEMBER 30, 1992**

RESEARCH

1. **FREQUENCY OF HCV-RNA IN ELISA AND RIBA II ANTIBODY REACTIVE DONOR SERA.**

The presence of antibodies to hepatitis C virus (HCV) is evidence of prior and/or ongoing exposure to HCV infection. Antibody testing, however, does not necessarily indicate whether a blood donation contains HCV and is potentially infectious. Levels of HCV-RNA in serum and liver, in general, appear to parallel disease activity, as indicated by increased serum alanine aminotransferase (ALT) levels. In this study, the frequency and overall concordance of appearance of HCV-RNA, using PCR, and antibody, using ELISA and RIBA II, in donor sera will be undertaken.

2. **ESTABLISHMENT OF A PRIMARY CELL CULTURE FROM CHIMP LIVER BIOPSIES TO GROW AND MAINTAIN HCV**

The genome of hepatitis C virus (HCV), the etiologic agent of most post-transfusion non-A, non-B cases is a positive stranded RNA virus. The susceptibility of chimpanzees to non-A, non-B hepatitis has enabled collection of serial blood and liver biopsies from different stages of infection. However, as yet no complete virions have been isolated. One of the likely reasons for the failure to demonstrate these agent(s), or to detect virus-like antigens, in serum, may be the very limited degree of viral replication in the infected host, as suggested by low infectivity titer of most plasma. We anticipate that the development of a primary culture of liver cells might prove susceptible.

3. **GENETIC DIVERSITY OF HEPATITIS C VIRUS: A LONGITUDINAL STUDY OF PERSONS WITH ONE OR MORE STRAINS**

Hepatitis C virus (HCV) is the etiologic agent in the majority of cases of transfusion associated hepatitis (TAH) in the United States. HCV disease can also be contracted by intravenous or percutaneous blood exposure such as intravenous drug abuse, and occupational exposure to blood borne pathogens.

Initiatives to reduce the incidence of the disease include donor blood testing and research on vaccines. The former has already resulted in a reduction of the risk of transmission of HCV by blood. Development of the latter is hampered by the genetic diversity of the virus. As evidence of this diversity accumulates, it appears that multiple strains of the virus exist (at least 4 of which have been described in the U.S., Japan and Eurasia), and evolutionary

changes occur in strains over time. The latter is the subject of this study.

The investigators propose to obtain stored sera of 30 to 50 persons who have been referred for testing for HCV on more than one occasion. Positive screening tests are often repeated within six months. A comparative analysis of preselected structural regions in the genome for variation between the first and subsequent samples will be done on sample with positive confirmatory tests. It is hypothesized that the observed changes will not be random. A better understanding of the replication process will increase the possibility for successful vaccine development and/or treatment.

REGULATORY

1. Standard operating procedures and checklists continue to be developed to better standardize product reviews. These documents, for internal use in the Laboratory of Hepatitis, are intended to both formalize review content and policies, and thus, should lessen the impact of personnel turnover in the Laboratory.

Publications

1. Nedjar S, Mitchell F, Biswas R. Enzymatic co-amplification and simultaneous detection of hepatitis B virus and hepatitis C virus-specific genomic sequences in donor sera. Transfusion, vol. 31(1991) 58s.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 BG 04008 02 LH

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 CHARACTERS OR LESS. Title must fit on one line between the borders.)

Simultaneous Detection of HBV and HCV Genomic Sequences by PCR

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title.)

Sayah Nedjar, Ph.D., DTS, LH

F. Mitchell, DTS, LH

R. Biswas, M.D., DTS, LH

COOPERATING UNITS (if any)

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Laboratory of Hepatitis, Division of Transfusion Science

SECTION

INSTITUTE AND LOCATION

FDA, CBER, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A sensitive and specific two stage polymerase chain reaction (PCR) method was developed for the simultaneous amplification and detection of specific genomic sequences of hepatitis B virus (HBV) and hepatitis C virus (HCV) in donor sera. Initially, HCV-RNA was reverse transcribed to cDNA. This cDNA, and DNA from HBV, were then co-amplified by using primer pairs derived from conserved regions of HBV and HCV nucleotide sequences. The specificity of PCR products was confirmed by liquid hybridization analysis using ³²P-end labeled oligomer probes specific for HBV and HCV nucleotide sequences. Independent sets of sera, positive and negative by PCR for either HBV-DNA or HCV-RNA, were used as controls. Nine donor sera, antibody reactive for both HBV (HBsAg and anti-HBc) and HCV (anti-HCV), were tested. Our assay detected HBV and HCV-specific genomic sequences in 9/9 sera reactive for both HBV and HCV antibodies. The ability to co-amplify and detect HBV and HCV sequences, non-isotopically may be particularly useful in the clinical laboratory setting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG 04002 03 LH

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (20 CHARACTERS OR LESS. Title must fit on one line between the borders.)

Hepatitis C and Therapeutic Immunoglobulin Product Safety Study Chimpanzee

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title)

Robin Biswas, M.D., DTS, LH;

F. Mitchell, DTS, LH

L. Wilson, DTS, LH

D. Tankersley, DH, LPD

J. Finlayson, Ph.D., DH

S. Nedjar, DTS, LH

COOPERATING UNITS (if any)

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FDA, CBER, Bethesda, MD 20982

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The safety of immune globulin products, in regard to hepatitis C (HC), manufactured from anti-HCV depleted plasma pools, is being studied in chimpanzees and is still in progress. As reported last year, plasma from anti-HCV negative Source Plasma donations was pooled and fractionated into intravenous immune globulin (IVIG). Samples from the unprocessed plasma pool were inoculated into 2 chimpanzees. These animals became infected with HCV as demonstrated by the development of anti-HCV, ALT elevations and the detection of HCV-RNA in the plasma. IVIG was then inoculated into 3 chimpanzees. If any of these animals had developed HC, it would have implied that screening for anti-HCV is deleterious to the safety of IVIG. If none of these animals had developed HC, it would suggest that screening plasma for anti-HCV will not adversely affect immune globulin safety. Twelve months after inoculation of IVIG had passed, none of the animals showed any evidence of hepatitis infection. This data was used as the basis to recommend the screening of source plasma for anti-HCV. Subsequently two animals were challenged with known infectious human plasma to ensure the capability of infectivity, and a third animal remained unchallenged and serves as a control. One of the animals that was challenged with HCV has seroconverted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 BG 04010 01 LH

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronology of Appearance of HCV-RNA and Antibodies in HCV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title.)

Sayah Nedjar, Ph.D., DTS, LH

F. Mitchell, DTS, LH

R. Biswas, M.D., DTS, LH

COOPERATING UNITS (if any)

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Laboratory of Hepatitis, Division of Transfusion Science

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TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The sequence of appearance of HCV-RNA and of the different antibodies to hepatitis C virus (HCV) epitopes following experimental infection of two chimpanzees with HCV, have been investigated. The chimps were infused with unprocessed, pooled plasma from donations that were non-reactive for anti-HCV. Serum samples were obtained prior to infusion, and then weekly subsequent to infusion. These samples were tested for anti-HCV, HBsAg, anti-HBs, anti-HBc and alanine aminotransferase (ALT). Serum samples were also tested for the presence of HCV-RNA by polymerase chain reaction assay (PCR) and for antibodies to c100-3, C22-3, c33c and 5-1-1 by the immunoblot, RIBA II test. The two chimpanzees showed different patterns in the sequential and temporal appearance of HCV-RNA and of antibodies to the different HCV epitopes. HCV-specific genomic sequences were detected in serum within one week after infusion in one animal and 9 weeks in the other chimp. Elevations in ALT values were observed 3 weeks subsequent to infusion in both animals.

ANNUAL REPORT OF THE LABORATORY OF RETROVIROLOGY,
DIVISION OF TRANSFUSION SCIENCE,
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH,
FOOD AND DRUG ADMINISTRATION

October 1, 1991 through September 30, 1992

The Laboratory of Retrovirology in the Division of Transfusion Science is engaged in research related to retroviruses that may be applied toward improving the safety of the blood supply through donor testing and product manufacturing. The scientific component of the laboratory is presently organized into sections of HIV Immunochemistry, HIV Molecular Genetics, HTLV-I/II and Product Testing. Current projects involve HIV-1, HIV-2, HTLV-I and HTLV-II. The laboratory also develops reference standards such as serum panels, and conducts scientific studies to define the basis for public health policy decisions related to donor suitability criteria and retroviral testing. Regulatory work in the laboratory involves reviewing IND's and PLA's for AIDS-related and other retroviral diagnostic tests, including those with potential use to screen donated blood or plasma. Additionally, there is a program of lot release testing for licensed kits. The laboratory also responds ad hoc to issues related to transfusion transmission of non-viral infections.

The Laboratory was the subject of a site visit on September 24, 1991 with additional presentations and discussion at a meeting of the Blood Products Advisory Committee on December 13, 1991. A report of the site visit team has been provided to the Director, Office of Biologics Research, Center for Biologics Evaluation and Research.

The following is a summary of scientific activities in FY'92:

I. Projects Related to HIV and HTLV-I/II Diagnostic Testing and the Safety and Efficacy of Blood Products

A. Pre-Clinical and Clinical Studies

Several new and on-going studies were performed to assess the status of retroviral infection in study subjects or blood products or to clarify the meaning of retroviral test results. Some of these studies also involved the use of novel diagnostic methods.

1. Polymerase chain reaction (PCR), antibody profile and antigen level were used to monitor the efficacy of passive immunotherapy with HIV hyperimmune plasma in AIDS patients.
2. PCR was used to exclude HIV infection in a seronegative subject with aggressive Kaposi's Sarcoma (KS) who was identified in Spain. The negative result has implications for a possible

non-HIV etiology of the aggressive form of KS.

4. In vitro studies were performed to investigate the kinetics of HIV-1 inactivation during refrigerator storage of red cells in different anticoagulant solutions. These studies were undertaken to clarify viral risks that could be related to a reduced dating period for stored red cells.
5. PCR and antibody assays were used to characterize the status of infection and virus type in individuals thought to be infected with HTLV-I/II. Additionally, whole virus based ELISA tests have been developed which permit serotyping of antisera to HTLV-I/II. The findings were used to pedigree reference sera for an HTLV-I and HTLV-II control panel.
6. A T-cell line containing HTLV-II was previously established from a patient with aplastic anemia. Efforts are ongoing to characterize the infecting virus both biologically and at the genetic level.
7. The possibility of seronegative HIV infections is being investigated by PCR and by cellular immune responses to HIV antigens in a high risk population of IV drug abusers.
8. Following reports of increased test kit false reactivity for HIV, HTLV and HCV in the flu season, all licensed retroviral kits were surveyed for the susceptibility to such false reactions. Also, using pre- and postvaccination sera, a study was carried out to determine the relationship of recent vaccination against influenza or hepatitis B to the occurrence of falsely reactive tests in different kits.
9. A statistical analysis was done to determine the relative performance on lot release of the licensed screening tests for antibodies to HIV-1. The results will be useful in designing an updated control panel for HIV-1.
10. In vitro studies are ongoing which relate to potential use of Brucella abortus antigen as a vaccine carrier.

B. Laboratory Development of Screening and Diagnostic Assays

Investigations have continued into the development of more rapid, sensitive and specific assays for HIV, HTLV,

and other blood transmitted agents.

1. PCR based detection has been developed for HIV-2.
2. Rapid and quantitative assays have been developed for detection of HIV-1 infectivity in cell culture. An assay based on detection of TAT has been adapted to measure strain specific neutralization of cell-associated and cell-free virus inocula.
3. Using an HIV infectivity assay based on detection of TAT, monoclonal human and murine antibodies were evaluated for strain specific neutralization as part of a WHO/NIH initiative related to development of vaccines and therapeutics for HIV disease.
4. Studies continue on the development of rapid and quantitative assays for HIV-1 specific DNA and RNA in serum and in supernatants of infected cells in culture. PCR based detection of HIV in other tissues has been investigated, including a method for in-situ PCR. A technique has been developed for PCR following antibody mediated viral capture onto magnetic beads.
5. A novel RIPA was developed for characterization of antibodies to HIV-2. This assay has been used to pedigree HIV-2 positive sera as control reagents in a lot release panel.
6. Murine monoclonal antibodies to HTLV-I have been made which are being used to develop a fine characterization of HTLV-I antigenic determinants that may lead to improved diagnostic tests.

C. Basic Research on Retrovirus Infection

Several ongoing projects are directed toward the study of HIV infection including latency, activation and drug suppression. Viral tropism and virulence factors are also under study.

1. The antiviral activity and underlying genetic events continue to be explored for a variety of compounds including AZT, interferon alpha, ddC and ddI. Many other compounds are under study including foscarnet, amphotericin B and Nystatin, as well as inhibitors of HIV protease, integrase and reverse transcriptase. Newer compounds tested include agents which may inhibit virus binding to CD4 and antineoplastons (anti-cancer peptides).

2. Modified antisense oligonucleotides and triple-helix-forming oligonucleotides are being investigated for the ability to inhibit or regulate HIV gene expression in cell cultures. Antisense sequences have also been introduced into HIV infected cells by the use of retroviral vectors.
3. The potential usefulness of liposomes and immunoliposomes for intracellular delivery of antiviral compounds is under study. A wide variety of compounds, including known antiviral drugs, membrane active antibiotics, anti-cancer drugs and cytokines, have been encapsulated and studied for efficacy against HIV-1 in vitro.
4. The induction of HIV-1 from a latently infected cell line by chemical carcinogens and dietary substances has been under study. These studies have been extended to an investigation of the role of NFkB and TNFa in viral induction.
5. Cellular factors that may control HIV infection are under study including expression of cellular proto-oncogenes and cytokines.
6. Studies of virus tropism are ongoing including efforts to infect non-lymphoid cells such as neural cell lines and cervical cell lines in addition to previous studies on persistent HIV infection in mammary epithelial cell lines. Also, studies are ongoing of infection of lymphoid cells at different stages of differentiation.
7. Recombinant HIV-1/2 and HTLV-I/II viruses are being prepared in an effort to study the molecular basis for virulence and tropism of these retroviruses.
8. Studies have been done on C' mediated killing of HIV-2 infected cells.
9. The effects on HIV infection of lymphocyte stimulation with Brucella abortus antigens, IFN-gamma and IL-10 have been studied in vitro.

II. Other Scientific Research

1. An ongoing study is investigating the anti-tumor effect of antisense oligonucleotides directed against a tumor specific gene in colon cancer.
2. A study is ongoing to investigate the effect of

Amphiregulin (a cytokine resembling EGF) on mammary epithelial tumors.

3. Studies on the involvement of the FLG FGF-receptor in normal development and tumorigenesis, including evaluation of FGF receptor amplification as a prognostic factor in relevant cancers.

III. Contracts

Continuing contracts are in place which support FDA research and regulation:

1. An interagency cooperative agreement exists for support of on-going investigations by the Division of Transfusion Medicine, Clinical Center, NIH on the clinical significance of antibodies to HIV-1 in blood donors. In addition to yielding valuable information on donor screening and testing, this contract has established a repository of serial samples of serum, lymphocytes and viral isolates from HIV positive subjects and controls. The repository is being used as a resource for studying virus variation in the course of HIV disease and to develop reference reagents such as neutralizing antibodies.
2. A support contract for testing and storage of serum and plasma reagents is in place. This contract has been used for preparation of reference panels as well as other scientific support functions such as routine gene sequencing.

IV. Accomplishments in Regulation

A. Product Reviews and Product Testing

The Laboratory reviewed and brought to approval the first immunofluorescence assay for HIV-1 antibodies, two combination tests for antibodies to HIV-1 and HIV-2, a visually read colorimetric rapid assay for anti-HIV-1, and the fourth test for antibodies to HTLV-I. The Laboratory has also approved ten major product license amendments. Additionally, ten PLA's, six ELA's, one PMA and twelve license amendments are under current review. These include four combination tests for detection of antibodies to HIV-1 and HIV-2, one test for HIV-1 antigen, and one test for detection of anti-HTLV-I/II. Also under active review are 13 original IND's in FY'92 and 42 supplements. The Laboratory inspected manufacturing facilities at 13 locations, including three prelicense inspections. A panel for lot release testing of test kits for HIV-2 antibodies has been prepared for distribution to

manufacturers and a new lot release panel for HTLV-I is close to completion.

The Product Testing Section performed lot release testing of approximately 990 lots of licensed kits for detection of antibodies to HIV and HTLV. These kits include eight HIV-1 EIA's, two HIV-1/HIV-2 EIA's, one particle agglutination test for anti-HIV-1, one antigen detection test for HIV-1, three Western blot tests for anti-HIV-1, one antibody test for HIV-2, one test for HIV-1 antigen, and four anti-HTLV-I EIA's. Additional testing was performed on 15 kit lots of investigational test kits including immunofluorescence assays and rapid tests for anti-HIV-1, combination tests for antibodies to HIV-1 and HIV-2, and tests for anti-HTLV-I. Approximately 200 additional sample tests were performed on proficiency panels for HIV or HTLV provided by the Centers for Disease Control. An additional 2500 samples were run as part of an investigation into the extent and cause of the multiple reactive false positive phenomenon for HIV and HTLV kits. Another 3000 sample tests were performed in support of research related to development of reference panels.

B. Policy Development

Efforts of this laboratory have contributed to development of regulatory policies in several areas related to transfusion safety. These included extensive revisions of FDA's "Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products" which encompassed modifications to donor exclusion, implementation of HIV-2 testing including the use of combination tests for HIV-1 and HIV-2, and new recommendations for product recalls and recipient notification related to prior collections from a subsequently seroconverted donor.

The laboratory also developed recommendations on use of the newly approved immunofluorescence test for antibodies to HIV-1 and a clarification of FDA recommendations on donor deferral related to history of syphilis. Efforts are ongoing to address test result invalidation based on aberrant procedures or test results during donor screening, and possible FDA blood product recalls based on risk information learned subsequent to a donation.

Additionally, the laboratory has contributed extensively to the PHS Interagency Workgroup on Organ and Tissue Transplantation including an ongoing effort to develop "USPHS Recommendations for Prevention of Transmission of HIV Through Transplantation of Human Tissues and Organs."

C. Awards

Individuals in the laboratory were selected for an FDA Group Recognition Award for contributions to the success of Operation Desert Storm. Related efforts included consultation with the military about pseudomonas contamination of red cells which had been stored in the Arabian peninsula, discussions with DOD on the potential implications for donor suitability of exposure in the Persian Gulf area to a novel strain of *Leishmania tropica* which caused cases of visceral disease.

V. Plans for the Future

A. Research efforts directly in support of product regulation will include:

1. Extension of the lot release panel for HIV-1 to include more difficult samples for sensitivity of detection
2. Development of standard diagnostic PCR reagents for HIV-1, HIV-2, HTLV-I and HTLV-II
3. Development of reference immunoassays for antibodies to HIV-1, HIV-2, HTLV-I and HTLV-II, including RIPA and Western blot
4. Development of a RIPA assay for HTLV-I based on purified surface glycoproteins
5. Development of a PHS position on donor screening for HTLV-II and a control panel for HTLV-II antibodies. Continued refinement of HTLV-I and II control reagents for lot release.
6. Continued development of approaches to the control of transfusion-transmitted Chagas' disease, *Yersinia enterocolitica*, bacterial contamination in platelets and other rare infections.
7. Continued investigation of kit lot variations to establish a sound basis for expedited lot release.
8. Continued studies on the cause and significance of false positive EIA tests for viral markers.
9. Investigation of chemical and physical methods for inactivation or removal of viruses from blood and blood products.

B. Other Research

Ongoing research will be sustained in all areas of

investigation that are described in Sections I. and II. Specific research objectives will include:

1. Continued development of more sensitive, rapid and quantitative assays for nucleic acid based detection of HIV-1 and HIV-2. Optimization of primers and probes to distinguish HIV-1 and HIV-2 infections. Development of sensitive and specific non-isotopic systems for detection of HIV-1/2 and development of in-situ PCR for tissue, including slide-based and FACS based tests.
2. Characterization of the role of cytokines and cellular genes in the regulation of HIV-1/2 gene expression.
3. Identification of novel anti-HIV agents and elucidation of their mechanism of action.
4. Development of novel antisense and triple helix oligomers and retroviral vector based constructs expressing antisense RNA to modulate viral gene expression.
5. Identification of pathogenic determinants of HIV-1 by construction of recombinant viruses and generation of less cytopathic variants that may be of use in vaccine development.
6. Continued development of modifications to PCR including co-amplification for detection of multiple viruses and optimization of nonisotopic methods. Development of alternative methods of gene amplification including LCR and 3SR techniques.
7. Continued studies on the induction of HIV-1 from latently infected cells by chemical carcinogens, hormones, drugs of abuse and cytokines. Identification of factors that regulate viral latency.
8. Identification of cellular factors that regulate viral gene expression by infection of cell lines that support virus replication to different extents. Further studies on the early and late cellular and viral events that occur in these target cells. Infection of non-lymphoid cell lines (neuronal, epithelial) with HIV-1 and HIV-2 to study the cellular factors involved in virus expression or restriction in these tissues.

9. Analysis of cellular and viral events in attempted HIV-1 infection of primary cells isolated from whole blood by cell sorting.
10. Clinical collaborations to validate diagnostic and prognostic markers in HIV disease in both natural history and intervention studies including passive immunization and antiviral drug therapy.
11. Characterization of virus variation in the course of HIV infection in closely monitored patients.
12. Continued investigation of "silent" HIV infections including IV drug users and their sexual partners and hemophiliacs and their partners.
13. Continued optimization of neutralization assays for HIV-1 and their application in vaccine and therapeutics development and in trials.
14. Expanded use of FACS and IFA to identify and quantitate lymphocyte subsets susceptible to HIV-1 infection, and to monitor virus infection in vivo.
15. Continued development of liposomes as a drug delivery vehicle for HIV infected cells.
16. Purification of surface glycoproteins of HIV-1, HIV-2 and HTLV-I for the purpose of characterizing their role in cell infection and tropism. Investigation into the mechanism of HIV-1 cell binding, fusion and internalization.
17. Continued studies of the early antibody responses to HIV infection.
18. Studies of neutralizing antibody phenotypes during the course of HIV infection.
19. Studies of the neutralization epitopes of HIV.
20. Gene sequence analysis of DNA from HTLV-I and HTLV-II isolates obtained from blood donors and from isolated populations of indigenous Americans.
21. Continued characterization of a human HTLV-II containing cell line and on the possible etiologic role of HTLV-II in aplastic anemia.
22. Fine characterization of HTLV-I and HTLV-II antigens through the use of monoclonal antibodies and through the expression of recombinant proteins

and synthetic peptides.

23. Studies of HTLV-II in IV drug users to determine the rate of HTLV-II infections missed by screening tests for HTLV-I.
24. Continued studies on the involvement of the FLG FGF-receptor in normal development and tumorigenesis. Evaluation of FGF receptor amplification as a prognostic factor in relevant cancers.
25. Studies of the use of retroviral vectors to express proteins important in the therapy of blood disorders.
26. Continued studies on the viability of HIV-1 in refrigerator stored red cells prepared in various additive solutions.
27. Investigations of the immunogenicity in animals of HIV proteins conjugated to Brucella abortus antigen.

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16. Soriano V, Hewlett I, Friedman-Kien A, Huang Y, Tor J, Epstein J. Definitive exclusion of HIV infection in a Kaposi's Sarcoma (KS) bisexual man: suggestions of a pathogenic model for KS, *Vox Sanguinis* 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06015-04(LR)												
PERIOD COVERED October 1, 1991 to September 30, 1992.														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Anti-HIV activity of novel antiviral agents														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Bharat Joshi</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">DTS/CBER</td> </tr> <tr> <td>Other: Indira K. Hewlett</td> <td>Section Chief</td> <td>DTS/CBER</td> </tr> <tr> <td>Sherwin Lee</td> <td>Bio. Lab. Tech</td> <td>DTS/CBER</td> </tr> <tr> <td>Jay S. Epstein</td> <td>Lab Chief</td> <td>DTS/CBER</td> </tr> </table>			PI: Bharat Joshi	Visiting Fellow	DTS/CBER	Other: Indira K. Hewlett	Section Chief	DTS/CBER	Sherwin Lee	Bio. Lab. Tech	DTS/CBER	Jay S. Epstein	Lab Chief	DTS/CBER
PI: Bharat Joshi	Visiting Fellow	DTS/CBER												
Other: Indira K. Hewlett	Section Chief	DTS/CBER												
Sherwin Lee	Bio. Lab. Tech	DTS/CBER												
Jay S. Epstein	Lab Chief	DTS/CBER												
COOPERATING UNITS (if any)														
LAB/BRANCH Lab. of Retrovirology														
SECTION Molecular Biology														
INSTITUTE AND LOCATION CBER/FDA														
TOTAL STAFF YEARS: 0.75	PROFESSIONAL: 0.60	OTHER: 0.15												
CHECK APPROPRIATE BOXES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The antiviral agents AZT, DDC and interferon alpha were evaluated for their effectiveness against HIV by PCR. Briefly, H9 and U937 cells were infected with cell-free HIV-1, after which the drugs were added into the culture at specified concentrations: AZT 50 ug/ml and rIFNα at 50 units/ml or in combination at the same concentrations. Genomic DNA and RNA were analyzed by PCR using primers to the gag region(SK38/39). Virus production was measured by RT and p24 antigen assays. Greater than 95% inhibition of HIV replication was observed with AZT and the combination of AZT and rIFNα from 3-4 days after infection using the p24 and RT assays in both H9 and U937 cells. Viral DNA and RNA synthesis were not significantly inhibited by rIFNα. Some inhibition of DNA and RNA was observed with AZT alone followed by a recovery of viral nucleic acid with time. More than 80% inhibition of viral DNA synthesis was observed with the combination of AZT and rIFNα. Viral RNA was undetectable in both infected H9 and U937 cells in the presence of AZT and rIFNα combined. DDC was similarly evaluated using infected H9 and U937 cells. Treatment with concentrations of 0.5 and 1uM resulted in greater than 95% inhibition of HIV DNA on both target cells. AZT and DDC alone at the higher concentration inhibited viral DNA and RNA synthesis in cells and in supernatant, but significant toxicity was noticed (60-70% viable cells). Combination treatment with either AZT or DDC and rIFNα at lower doses resulted in significant inhibition of HIV DNA and RNA synthesis in cells and RNA in supernatants, with minimal toxicity (95% viability) in the treated cultures. In other studies, a spectrum of compounds that inhibit gp120 binding to CD4 including aurin tricarboxylic acid, DO26 and DR79 were found to inhibit HIV replication as measured by p24 antigen values and DNA PCR. In other experiments, inhibitors ODC, tyrosine kinase, divalent metal chelators, integrase, protease, RT, DNA primase and gyrase are being evaluated in vitro for anti-HIV activity. The molecular mechanism of this inhibitory effect will be studied by analyzing the expression of various 1 host genes in response to drug treatment. </p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 BG06018-04(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992.		
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Rapid and quantitative detection by PCR of HIV-1 specific DNA and RNA.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Bharat Joshi, Visiting Fellow DTS/CBER Others: Indira K. Hewlett, Section Chief DTS/CBER Sherwin Lee, Bio. Lab. Tech DTS/CBER Jay S. Epstein, Lab Chief DTS/CBER		
COOPERATING UNITS (if any) 		
LAB/BRANCH Retrovirology		
SECTION Molecular Biology		
INSTITUTE AND LOCATION CBER/FDA		
TOTAL STAFF YEARS: 0.50	PROFESSIONAL: 0.35	OTHER: 0.15
CHECK APPROPRIATE BOXES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A rapid and sensitive PCR assay for detection of HIV-1 specific DNA and RNA in culture supernatants and viral RNA in serum was developed. Culture supernatant from H9 and U937 cells infected with 100ng of p24 antigen/ 50 million cells was analysed on a daily basis for viral p24 antigen, DNA and RNA. For analysis of DNA, culture supernatant was heated in the presence of nonanionic detergent and an aliquot was used for PCR. Primer pairs from the gag, env and nef regions of the viral genome were used for co-amplification. RNA extraction was achieved from samples by a single step procedure of guanidine thiocyanate/ phenol/ CHCl₃ extraction and precipitation with isopropanol. PCR products were analyzed by slot-blot or liquid hybridization with radiolabelled oligonucleotide probes followed by PAGE and autoradiography. By this method 1-10 copies could be detected using the 8E5 cell standard. Viral RNA present in 100 ul of serum from an AIDS patient or an equivalent of 0.2 pg of p24 antigen could be detected in culture supernatant from infected H9 cells. Viral DNA and RNA were detected in culture supernatant of infected cells at 1 day post-infection while significant levels of p24 antigen were not detected until 2-3 days at the dose of virus used. In the culture supernatants from the cells treated with AZT, no viral RNA or DNA was detected at 3 and 7 days post infection and treatment suggesting that PCR on supernatants may be useful to monitor antiviral activity. No activity was observed in control samples of serum or uninfected H9 cell culture fluid. Our result suggests that PCR on supernatants may be useful in monitoring co-cultures from infected individuals or patients undergoing therapy, as well as to monitor infection in in vitro studies. The methodology has been applied successfully for detecting virus from other body fluids such as urine, sweat and saliva. We are currently extending its application for more rapid detection by using non-isotopic systems either by using chemiluminescent or fluorescent primers/ probes that would achieve single copy detection in conjunction with these techniques.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06021-03(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infection of non-hematopoietic cells with HIV-1 and HIV-2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira K. Hewlett, Section Chief DTS/CBER

Other: Alonso Heredia, Visiting Fellow DTS/CBER
 Uday Muppala, Visiting Fellow DTS/CBER
 Sherwin Lee, Bio. Lab. Tech DTS/CBER
 Jay S. Epstein, Lab. Chief DTS/CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV-1 has been known to infect a variety of non lymphoid cell lines, where the mode of entry has been postulated to involve an additional or an independant receptor or a second messenger-like molecule. The extent of viral replication in these cell lines is vastly different from productive to abortive infections suggestive of host dependant controls on viral replication. These model systems are ideal for identification of cellular and viral factors involved in replication. Also, in some instances, certain epithelial cells from patients (e.g colon, cervical etc) have been shown to harbor viral nucleic acid. Recent epidemiologic studies suggest that some transmission to the fetus may occur in utero and may involve infection of non lymphoid cell types. We have initiated studies to explore mechanisms of active or possibly abortive viral infections in these cell types. The target cells we have chosen to work with are neuroblastoma cell lines SKNSH, SKNMC, a human embryonic cell line, L132, two cervical cell lines SiHa and C33A, two uterine cell lines HS 825T and HS 258T and a vaginal cell line HS 769 Vg. These cell lines have been infected with HIV-1 and the course of infection is being monitored. The kinetics of viral replication assessed by synthesis of viral DNA, RNA and proteins is being analyzed in these cell lines. Cellular factors such as NFkB, NFAT, c-fos/jun, kinases, methylases and cytokines that may govern the different extents to which viral replication occurs in these different cell lines will be investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 BG06022-03(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992.

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Induction of HIV-1 from latently infected cell lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bharat Joshi, Visiting Fellow DTS/CBER

Other: Indira K. Hewlett, Section Chief DTS/CBER
 Sherwin Lee Bio. Lab. Tech DTS/CBER
 Gary Riordan Bio. Lab. Tech DTS/CBER
 Jay S. Epstein Lab. Chief DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Lab. of Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

0.75

PROFESSIONAL:

0.50

OTHER:

0.25

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The U1 cell line, derived from a chronically infected U937 culture harbors a latent HIV genome which can be induced to synthesize virus in the presence of TPA, TNF α , IL-6, okadaic acid and some other cytokines. Various chemical carcinogens and environmental mutagens were examined for their ability to induce virus from U1 cells with the objective to identify risk factors of physiologic relevance that may modulate disease progression in AIDS. In the first phase of the experiment, the cells were treated with carcinogens and harvested at different time points for measurement of p24 and RT activity from supernatants. Cell pellets were harvested for DNA and RNA preparation. Among these, benzo(a)pyrene (BOP) and alpha hexachlorocyclohexane (HCH) were found to be upregulating the virus production more than 18 and 12.5 fold after six days of treatment. TPA served as the positive control in the experiment. Enhanced levels of HIV RNA were observed in BOP and HCH treated cultures. A ten fold increase in virus production was observed using electron microscopy. In order to understand the mechanism of virus induction, experiments were carried out to analyze the induction of NF-kB protein, or the production of cytokines like TNF α or phosphorylation of kB complex. It is interesting to note that BOP and HCH both induce NF-kB protein as early as 2 hours of treatment and the effect persists for 2 days. Treated cell free supernatants showed less elevated TNF α activity after 24 hours than TPA treated supernatants. In case of BOP treated cells, phosphorylation of the I κ B complex by Protein Kinase C is observed reflected by generation of enhanced NF-kB activity for overall up-regulation of the virus. Other environmental mutagens that were analyzed and showed more than 5 fold viral induction included cigarette smoke constituents such as NNK, NNN, dimethyl nitrosamine (DMNA) and aflatoxin. Nicotine, NNN, NNK and DMNA were found to induce NF-kB protein at different time points. The involvement of PKC activity was observed in cells treated with nicotine, NNK and DMNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG 06026-05(LR)												
PERIOD COVERED October 1, 1991 through September 30, 1992														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Optimization and quantification of markers of HIV infection and replication														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; padding: 5px;">PI: S. J. Geyer</td> <td style="width: 33%; padding: 5px;">Senior Staff Fellow</td> <td style="width: 33%; padding: 5px;">DTS/CBER</td> </tr> <tr> <td colspan="3" style="padding: 5px;">Others:</td> </tr> <tr> <td style="padding: 5px;">N. Neiger</td> <td style="padding: 5px;">Biologist</td> <td style="padding: 5px;">DTS/CBER</td> </tr> <tr> <td style="padding: 5px;">J. S. Epstein</td> <td style="padding: 5px;">Laboratory Chief</td> <td style="padding: 5px;">DTS/CBER</td> </tr> </table>			PI: S. J. Geyer	Senior Staff Fellow	DTS/CBER	Others:			N. Neiger	Biologist	DTS/CBER	J. S. Epstein	Laboratory Chief	DTS/CBER
PI: S. J. Geyer	Senior Staff Fellow	DTS/CBER												
Others:														
N. Neiger	Biologist	DTS/CBER												
J. S. Epstein	Laboratory Chief	DTS/CBER												
COOPERATING UNITS (If any)														
LAB/BRANCH Laboratory of Retrovirology														
SECTION Immunochemistry														
INSTITUTE AND LOCATION DTS/CBER/FDA														
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The purpose of this research project is to identify markers and develop rapid quantitative methods for 1) detecting and quantifying low levels of infectious HIV in blood and blood products, 2) estimating viral burden in clinical material, 3) characterizing virus target cells and 4) distinguishing among various virus strains.</p> <p>Rapid assays for infectious virus have been developed in the laboratory based on the indicator T-cell line (H938) containing multiple integrated copies of HIV-1-LTR-chloramphenicol acetyl transferase (CAT). The indicator T-cells signal infection with quantitative increase in enzyme activity. The T-cell assay has been used to determine the half-life of HIV-1 in packed red blood cells (see Z01 BG 0699-02(LR), and has formed the basis for a neutralization assay to assess potency and efficacy of monoclonal antibodies (see Z01 BG 0631-03(LR)). A rapid reverse transcriptase assay developed under this project has been used to titer the representative strains of HIV-1 virus (IIIB, RF, Z, MN) used as inocula for the neutralization test system.</p> <p>Currently a monocyte indicator cell line containing stably integrated copies of the HIV-1-LTR-CAT construct is being characterized with representative strains of HIV-1 (RF, IIIB, Z, MN) and adapted for use as a neutralization target cell.</p> <p>The CAT signal produced in the indicator assays presently use 3H-coenzyme A as substrate. Alternative substrates which produce non-radioactive products, but which are equally sensitive, are under active investigation.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG 06031-03(LR)
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization and mapping of HIV neutralizing antibodies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: S. J. Geyer	Senior Staff Fellow	DTS/CBER
Others:		
N. Neiger	Biologist	DTS/CBER
J. S. Epstein	Laboratory Chief	DTS/CBER
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Retrovirology		
SECTION Immunochemistry		
INSTITUTE AND LOCATION DTS/CBER/FDA		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The present study was undertaken to develop a rapid, quantitative, <u>in vitro</u> system for evaluating antiviral agents, neutralizing antibodies and various cytokine effects on HIV-1 replication. A system was developed to simultaneously test the effects of various agents on the replication of 1) representative strains of HIV, 2) virus presented cell-free or cell-associated, and 3) HIV transmitted to specific target cells, particularly T-cells and monocytes.</p> <p>The test system uses indicator T-cells (H-938) containing stably integrated copies of the HIV-1-LTR-CAT (chloramphenicol acetyl transferase) gene which respond to virus infection with increased CAT activity. For virus inocula, H-9 T-cells were similarly infected with HIV-1 (IIIB, MN, RF, or Z). Infected cells and culture supernatants were cryopreserved for use as cell-associated and cell-free virus inocula. Virus inocula for testing neutralizing antibodies were chosen to produce a 200-fold increase in CAT activity in indicator cells (1.4×10^4 per well) cultured for 3 days.</p> <p>In conjunction with the NIAID/WHO Antibody Serologic Project (ASP), the assay was used to evaluate the potency and strain specificity of controls and purified monoclonal antibodies (MAbs, 7 human, 7 mouse) directed toward V3, V2 gp41, and CD4 binding regions of HIV-1 gp120. There were 20 labs participating in the ASP study and the novel "LTR-CAT" target cell assay was among the most sensitive and specific neutralization assays in the study. Results showed that human MAbs tended to be more broadly neutralizing than mouse MAbs, and MAbs directed toward the V3 or gp41 epitopes of HIV-1 tended to be more potent than those directed toward the CD4 binding region of gp120. In addition, neutralization of cell-associated inocula required 1-2 logs higher MAB concentration than cell-free inocula, with one exception. These results have implications for therapeutics under consideration for licensure and vaccine development and were presented at UCLA Symposium on "Prevention of Treatment of AIDS" and are included in an abstract for "Modern Approaches to Vaccines" meeting at Cold Spring Harbor, NY., September 16-20, 1992.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06038-02(LR)

PERIOD COVERED

October 1, 1991 to September, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular factors that control viral expression and regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira K. Hewlett, Section Chief DTS/CBER

Other: Bharat Joshi, Visiting Fellow, DTS/CBER

Gary Riordan, Bio. Lab. Tech DTS/CBER

Alonso Heredia, Bio. Lab. Tech DTS/CBER

COOPERATING UNITS (if any)

Michael Norcross, Sr Staff Fellow DCB/CBER

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unruled type. Do not exceed the space provided.)

We are investigating the role of cellular factors that mediate signal transduction in the regulation of viral gene expression. We analyzed the expression of the cellular oncogenes c-fos, c-myc and v-src (a tyrosine kinase expressed in T-cells) in human peripheral blood lymphocytes (PBLs) and H9 and U937 cells following infection with HIV-1. Quiescent PBLs were infected with HIV-1 using serum-free medium in the presence or absence of IL-2. At various times after infection DNA and RNA were analyzed for the presence of viral and oncogene sequences and cells were fixed for analysis of antigen by flow cytometry. A 20 fold increase in c-fos RNA and a 4-5 fold increase in c-fos antigen was observed between 2 and 22 hours post-infection. This increase was inhibited by actinomycin D. In these same cells, HIV-1 sequences were first detected in genomic DNA at 2 hours and viral transcripts between 2 and 4 hours post-infection. Our preliminary results suggest that enhancement of the expression of some of these cellular oncogenes may play a role in regulating viral replication in infected cells. Similar studies are in progress with HUT-78 and CEM cells infected with HIV-2. Experiments evaluating the role of transfected c-fos and c-jun (another cellular oncogene) in the transactivation of the HIV genome are in progress. Gel retardation assays and UV cross linking studies are being performed to study these factors. We also plan to study the effects of anti-HIV agents on cellular kinases in HIV-1 and HIV-2 infected cells. These studies will enable us to further define the stages of viral replication at which these gene products may function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06039-02 (LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Efficacy of hyperimmune plasma in AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Indira K. Hewlett, Section chief DTS/CBER Other: Jay S. Epstein, Lab. Chief DTS/CBER Sherwin Lee, Bio. Lab. Tech DTS/CBER		
COOPERATING UNITS (if any) Josh Levy, Hemacare Corporation		
LAB/BRANCH Retrovirology		
SECTION Molecular Biology		
INSTITUTE AND LOCATION DTS/CBER/FDA		
TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.20	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Hyperimmune plasma was collected from healthy, HIV-1 infected individuals with high titers of neutralizing antibodies and administered to 50 AIDS patients. All patients were monitored for viral antibodies, antigen and viral nucleic acid by PCR. Peripheral blood mononuclear cells (PBMCs) and sera were collected from these individuals and from a group of placebo treated individuals. Viral load is being quantitated by DNA and RNA PCR on the lymphocytes and RNA PCR on serum. Preliminary results suggest that although there may be protection based on the levels of serum p24 antigen, viral DNA levels were not decreased.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06041-02 (LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibition of HIV-1/2 with antisense oligonucleotides and ribozymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira K. Hewlett, Section Chief DTS/CBER

Other: U. Muppala, Visiting Fellow DTS/CBER

G. Riordan, Bio. Lab. Tech DTS/CBER

M. Panersselvam, Bio. Lab. Tech DTS/CBER

Jay S. Epstein, Lab. Chief DTS/CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.50

PROFESSIONAL:

0.40

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Phosphorothioate modified antisense oligonucleotides (ASOs) have been designed complementary to various regulatory and structural genes of HIV-1. These ASOs are being evaluated for their anti-HIV activity using H9 and U937 cells as target cells. Antiviral activity is being assessed by p24 antigen and PCR. Preliminary results suggest that ASOs from the tat and the LTR regions were most effective in inhibiting virus expression. These ASOs are being packaged into liposomes and administered either singly or in combination with other ASOs or with AZT to target cells and their antiviral activity evaluated. Similar experiments are being performed with HIV-2. Oligonucleotides that allow the formation of ribozyme complexes or that interact with b-ribbon motifs on DNA are also being evaluated for their antiviral activity against HIV-1 and HIV-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06042-02(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Viral determinants of HIV-1 pathogenesis by recombinant viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> PI: Indira K. Hewlett, Section Chief </div> <div> DTS/CBER </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> Other: Gary Riordan, Bio. Lab. Tech Jay S. Epstein, Lab. Chief </div> <div> DTS/CBER DTS/CBER </div> </div>		
COOPERATING UNITS (if any) None		
LAB/BRANCH Retrovirology		
SECTION Molecular biology		
INSTITUTE AND LOCATION DTS/CBER/FDA		
TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.15	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The pathogenesis of HIV-1 and HIV-2 vary to a fair degree although the 2 viruses share some homology (40%) at the nucleic acid level. Specific viral determinants may govern the different pathogenicities of these viruses in their host cells. We have initiated studies involving the construction of recombinant viruses that have a single gene replacement of a given virus type. Target sequences that are currently being investigated are the LTR, tat, rev, env, gag and pol genes. These recombinants will be transfected into the host cell and the effects of these substitutions on viral replication will be studied.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 BG06043-02(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Modulation of HIV-1 expression by antisense RNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kimber L. Poffenberger, Sr. Staff Fellow DTS/CBER

OTHER: Indira K. Hewlett, Section Chief DTS/CBER
 Gary Riordan, Bio. Lab. Tech. DTS/CBER
 Sherwin Lee, Bio. Lab. Tech. DTS/CBER
 Jay S. Epstein, Lab. Chief DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.15

PROFESSIONAL:

0.05

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviral vectors carrying various target regions of the HIV-1 genome (gag, pol, tat, rev, vif, and nef) are being constructed using strong viral (CMV), and cellular (Beta-actin and tRNA) promoters to express the viral sequences. These sequences are in the antisense orientation relative to normal viral transcripts. These constructs will be stably integrated into H9 and U937 cells to evaluate the antiviral effects of high-level expression of particular antisense sequences. Those sequences having high antiviral activity will be further evaluated in HIV infection of primary human cells. In addition, promising antisense sequences will be dissected to identify the smallest possible regions having antiviral activity. This project has been put on temporary hold due to excellent results with other projects and insufficient manpower.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06044-02(LR)		
PERIOD COVERED October 1, 1991 to September 30, 1992				
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Modulation of HIV-1 expression with triple-helix-forming oligonucleotides				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Kimber L. Poffenberger, Sr. Staff Fellow DTS/CBER				
<table style="width: 100%; border: none;"> <tr> <td style="width: 60%; border: none; vertical-align: top;"> OTHER: Indira K. Hewlett, Section Chief Gary Riordan, Bio. Lab. Tech. Sherwin Lee, Bio. Lab. Tech. Jay S. Epstein, Lab. Chief </td> <td style="width: 40%; border: none; vertical-align: top;"> DTS/CBER DTS/CBER DTS/CBER DTS/CBER </td> </tr> </table>			OTHER: Indira K. Hewlett, Section Chief Gary Riordan, Bio. Lab. Tech. Sherwin Lee, Bio. Lab. Tech. Jay S. Epstein, Lab. Chief	DTS/CBER DTS/CBER DTS/CBER DTS/CBER
OTHER: Indira K. Hewlett, Section Chief Gary Riordan, Bio. Lab. Tech. Sherwin Lee, Bio. Lab. Tech. Jay S. Epstein, Lab. Chief	DTS/CBER DTS/CBER DTS/CBER DTS/CBER			
COOPERATING UNITS (if any)				
LAB/BRANCH Retrovirology				
SECTION Molecular Biology				
INSTITUTE AND LOCATION DTS/CBER/FDA				
TOTAL STAFF YEARS: 0.35	PROFESSIONAL: 0.25	OTHER: 0.1		
CHECK APPROPRIATE BOXES: <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Homopurine:homopyrimidine stretches of dsDNA can form triple helices with homopyrimidine oligonucleotides. Triple helical structures prevent transcription and translation across their sequences. We have designed several homopyrimidine oligonucleotides (ONs) which target specific HIV genes (gag, pol, tat, rev, nef) and modified them to prevent degradation by cellular nucleases. We tested the ability of these ONs to suppress HIV-1 infection of a lymphocyte cell line (H9). Infected cells treated with 10 to 50 nanomolar concentrations of ON produced from 50 - 90% less viral p24 antigen than control infected cells, in a dose-responsive manner. No cell toxicity or effect on cell doubling time was observed at these low concentrations of ON. DNA-PCR assays on treated cells showed decreased amounts of viral DNA relative to untreated cells. These results suggest that the ONs are functioning as triple helix-formers and could be an effective antiviral technology. We are continuing these studies with 1) primary human peripheral blood lymphocytes and 2) higher concentrations of ONs. </p> <p> This work has been accepted as an abstract for slide presentation at the 1992 ICAAC meeting to be held in October. </p>				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06045-02(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

HIV infection of hematopoietic cells at differing stages of differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kimber L. Poffenberger, Sr. Staff Fellow DTS/CBER

OTHER: Indira K. Hewlett, Section Chief

DTS/CBER

Jay S. Epstein, Laboratory Chief

DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

.05

PROFESSIONAL:

.05

OTHER:

0

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Pure populations of primary lymphocytes, monocytes, and dendritic cells will be separated from normal human blood via physical cell separation and FACS. Monoclonal antibody (MAB) markers defining various differentiated cell phenotypes will be used to identify and purify specific subpopulations of these cell types. The cells will be infected with HIV-1 and virus growth and gene expression will be evaluated by FACS, PCR, and other assays. Cell subpopulations which support HIV replication in vitro can then be assessed from HIV-seropositive blood to determine which subpopulations are viral reservoirs in vivo. Control experiments defining the antibodies, staining protocols and FACS parameters for HIV detection are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06047-02(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibition of HIV replication by Nystatin A and other agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Roy A. Blay, Ph.D. Senior Staff Fellow DTS/CBER

Sharon Geyer, Ph.D. Senior Staff Fellow DTS/CBER

Sheila M. Buck, Laboratory Tech., DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Nystatin A was compared with Amphotericin B and Foscarnet for their respective abilities to inhibit the replication of HIV-1 in H9 cells. Nystatin A and Amphotericin B are polyene macrolide antibiotics. Foscarnet is a trisodium phosphonoformate inhibitor of reverse transcriptase (RT). HIV-1 infected cells were cultured for seven days in the presence of 5-50 ug/ml concentrations of these three drugs, and RT activity and p24 antigen production were quantitated. Untreated HIV-1 infected H9 cells served as controls. Nystatin A was most effective inhibiting viral replication at 10 ug/ml, a concentration that did not affect cell viability. Nystatin A treatment inhibited RT activity by 95% and p24 production by 90% which was comparable to the level of inhibition mediated by Amphotericin B and Foscarnet. Western blot analysis of the HIV-infected H9 cells treated with these drugs did not detect any virus at the cellular level. These findings were further corroborated by indirect immunofluorescence studies using monoclonal anti-gp120 antibodies and FITC-conjugated secondary antibodies, and by polymerase chain reaction analysis using a 32P-labelled probe. These results suggest that Nystatin A merits attention as an antiviral drug for the treatment of HIV-1 infection. In vivo drug delivery by liposome encapsulation is currently under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06048-02(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Evaluation of antiviral activity of HIV-1 protease inhibitors in immunoliposomes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Sheila M. Buck, Laboratory Tech. DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief DTS/CBER

COOPERATING UNITS (if any)

Michael A. Ussery, Ph.D., Laboratory Chief, CDER/FDA

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Cerulenin, an inhibitor of fatty acid and sterol synthesis, has been shown to inhibit HIV replication in H9 cells by 50%. Cerulenin was highly toxic to cells at a 2 ug/ml concentration. The synthetic peptide analog of HIV protease, SKF-108922, was also shown to have an inhibitory effect on HIV replication. Both of these agents will be incorporated into immunoliposomes, and future experiments will focus on delivering these agents with greater specificity and less toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06049-02(LR)
PERIOD COVERED October 1, 1991, to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of free and antibody-targeted liposomal Nystatin A on HIV infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER Ronald E. Mayner, Section Chief, DTS/CBER Sheila M. Buck, Laboratory Tech., DTS/CBER Roy A. Blay, Ph.D., Senior Staff Fellow, DTS/CBER Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Retrovirology		
SECTION Immunochemistry		
INSTITUTE AND LOCATION CBER, FDA		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>Multilamellar immunoliposome vesicles containing Nystatin A were compared with free Nystatin A for toxicity to H9 cells and for antiviral activity in vitro. Preliminary results show that the liposomal Nystatin A is as active as free drug in the inhibition of HIV replication in H9 cells. Immunoliposome preparation is being modified to make it more specific for HIV-infected cells. The antiviral activity of these immunoliposome preparations will be further studied in in vitro cultures of H9 cells.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06051-02(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A novel sensitive radioimmuno precipitation assay system for detecting HIV-2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Sheila M. Buck, Laboratory Tech., DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a novel, sensitive and efficient radioimmuno precipitation assay (RIPA) which provides an alternative to the Western blot for characterizing antibodies against HIV-2. The assay is based on a radioiodinated antigen consisting of a soluble preparation of the NIH-2 (HIV-2) strain of 1000X purified virus spiked with purified recombinant HIV-2 gp105/110. Radiolabeled proteins were immunoprecipitated by immune human sera, even at the early stages of seroconversion. This method is more sensitive, has equivalent specificity, and is more efficient than Western blotting. Most importantly, the viral proteins labeled with Bolton-Hunter reagent are well suited to biochemical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06053-02(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HTLV I reference reagents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Martin Ruta, Ph.D.

Other: Ron Mayner, Chief Immunochemistry Section

Robert Sausville, technician

Jay Epstein, M.D. Lab Chief

COOPERATING UNITS (if any)

J. Lipka, Ph.D. Stanford Blood Bank

S. Fount, M.D. Stanford Blood Bank

LAB/BRANCH

Retrovirology

SECTION

HTLV

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.75

PROFESSIONAL:

0.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We analyzed sera reactive by HTLV I ELISA to pedigree reagents for HTLV I lot release panels. Sera were identified as HTLV I antibody positive by the Stanford Blood Bank. Lymphocytes from the positive patients were then analyzed to determine whether the patient was infected with HTLV I or II. PCR was performed using specific primer pairs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06054-02(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of a novel HTLV II cell line

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Martin Ruta, Ph.D.

Other: Mary Malarkey-Briggs

Robert Sausville

Gao Liu, Fogarty Fellow

Jay Epstein, M.D. Lab Chief

COOPERATING UNITS (if any)

Gary Armstrong, Division of Virology

Michael Clutch, DV

Carol Marcus-Sekura, Ph.D., DV

LAB/BRANCH

Retrovirology

SECTION

HTLV

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

1

PROFESSIONAL:

0.2

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We previously isolated a cell line (JR) from a patient with aplastic anemia that produces HTLV II. We are continuing to characterize this cell line and the virus that it produces in order to determine whether its association with aplasia is due to a unique characteristic of this isolate. We analyzed the proteins produced by JR cells by Western blot analysis of JR cells and purified virus and compared these results with standard HTLV II cells and viruses.

PCR based sequence analysis of DNA fragments amplified from JR cells were prepared and sequenced. These results show extensive homology between JR and known HTLV II isolates.

We are cloning JR virus. Eco RI fragments from genomic DNA of JR cells were prepared and cloned in a lambda vector. The library was screened using subclones of HTLV II Mo viral DNA. Positive clones were identified and partially sequenced. Our results indicate that there is extensive homology between JR and known HTLV II isolates. We are continuing our efforts to clone this virus.

We have analyzed sera from other aplastic anemia patients for antibodies to HTLV I/II. One additional reactive sample was detected. We are currently studying whether this person was infected with HTLV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06057-01(LR)
PERIOD COVERED October 1, 1991, to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoliposome-encapsulated interferon-gamma as an antiviral agent		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Roy Blay, Ph.D., Senior Staff Fellow, LR, CBER Mouna Selvam, Ph.D., Senior Staff Fellow, LR, CBER		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Retrovirology		
SECTION Immunochemistry		
INSTITUTE AND LOCATION CBER, FDA		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.18	OTHER: 0.02
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Liposomes have been used as a method of delivering a variety of drugs to specific cellular targets. The specificity of liposomal delivery has been further enhanced by the conjugation of specific antibody to the surface of the liposome to form an immunoliposome. The binding of the specific antibody to its target results in an enhanced concentration of drug in the target microenvironment and the liposome facilitates the entry of the drug into its respective cellular target. This specificity of action results in decreased toxicity and enhanced efficacy. Previous work has shown that encapsulation of various antibiotics such as Nystatin and Amphotericin B into liposomes has resulted in decreased yields of p24 antigen in cultures of HIV-infected H9 cells as compared to the activity of unencapsulated drug. This technique of encapsulating potential antiviral agents will be extended to a potent immunomodulatory cytokine with known antiviral properties, namely interferon-gamma (IFNg). Liposomes or immunoliposomes (liposomes conjugated to anti-HIV antibody) containing IFNg will be incubated with HIV-infected H9 cells for seven days at which time the supernatants will be harvested and assayed for p24 production. The efficacy of the encapsulated drug (IFNg) will be compared with that of the unencapsulated drug. If results are promising, that is, encapsulated IFNg does decrease viral yield, then these liposomes may be used in cultures of lymphocytes isolated from HIV-infected individuals to see if similar antiviral activity occurs in a primary cell culture. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06058-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of IL-10 on replication of HIV-1 in H9 cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Roy Blay, Ph.D., Senior Staff Fellow, LR, CBER

COOPERATING UNITS (if any)

Basil Golding, M.D., Senior Investigator, LCB, CBER

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.09

OTHER:

0.01

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Human interleukin-10 (IL-10) is a poorly characterized cytokine that is capable of inhibiting interferon-gamma (IFN γ) and granulocyte-macrophage colony stimulating factor production by mitogen-activated human peripheral blood mononuclear cells (PBMC). Human IL-10 has the same biological effects as murine IL-10 on murine cell targets. These effects include stimulating the proliferation of murine mast cells, increasing survival and expression of MHC Class II antigens on murine B cells, inducing the growth of peripheral T cells, and serving as a cytotoxic differentiation factor. With respect to the present study, the most relevant activities of human IL-10 are its abilities to inhibit IFN γ production and T cell proliferation in response to mitogens. What role, if any, that IL-10 has in the course of HIV-1 infection is unknown. It is possible that the suppressive activity of IL-10 upon IFN γ production may result in enhancement of HIV replication given the antiviral properties of IFN γ . Previous work has shown that H9 cells demonstrate evidence of productive HIV-1 infection seven days post-infection using p24 levels as an indicator of infection. The addition of IL-10 to such cultures at varying doses and times post infection should determine whether IL-10 has an effect on viral replication. Additional proliferation studies would indicate whether the H9 cell line is subject to the anti-proliferative properties of IL-10. Further studies using experimentally infected human peripheral blood lymphocytes may provide information on the role of this cytokine in the suppression of IFN γ production, T cell proliferation, and the enhancement of viral replication in a test system in which the presence of other cytokines such as IL-2 and IL-4 may interact with IL-10.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06059-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Kinetics of HIV-1 infection in H9 cells treated with Brucella abortus and IL-2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Roy Blay, Ph.D., Senior Staff Fellow, LR, CBER

COOPERATING UNITS (if any)

Basil Golding, M.D., Senior Investigator, LCB, CBER

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.18

OTHER:

0.02

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Previous investigations have shown that treatment of human peripheral blood lymphocytes (PBLs) from both uninfected and HIV-infected individuals with Brucella abortus (BA) and interleukin-2 (IL-2) results in the production of interferon-gamma (IFN γ) in a synergistic fashion. IFN γ is a pleiotropic lymphokine that has important immunomodulatory and antiviral activities; therefore, agents that induce the production of IFN γ may be of value for inclusion in the development of human vaccines for some viral diseases. BA, or the lipopolysaccharide component of BA (BA-LPS), has been shown to induce IFN γ and interacts synergistically with IL-2 to produce enhanced amounts of IFN γ . Because BA or its LPS component has potential for use as a carrier in the development of a human vaccine, we are investigating the ability of BA to influence viral replication in a human cell line (H9) permissive for HIV-1 infection. A cell line is being used because previous work has shown that freshly isolated human PBLs are resistant to HIV infection and do not provide a suitable model for study. Initial experiments have shown that H9 cells show evidence of HIV-1 replication within seven days of infection. BA or BA-LPS will be added to H9 cell cultures infected with HIV-1 and cultured for seven days. Supernatants of these cultures will be assayed for p24 as an index of viral replication. IL-2 will be added to some of these cultures to determine whether or not there is any synergistic activity between BA and IL-2 in enhancing viral replication. Our previous work has shown that BA, or the combination of BA and IL-2, is capable of eliciting IFN γ from the PBLs of HIV-infected individuals at different stages of disease. If BA is to be considered for use in a human vaccine, then the studies outlined here are necessary to distinguish between the IFN γ -inducing properties of BA and any possible upregulatory effects of BA on viral replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06060-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Performance studies of retroviral antibody detection tests

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald E. Mayner, Sect. Chief Immunochemistry, CBER

Other: Mouna P. Selvam, Staff Fellow, DTS/CBER

Charles Roberts, Sect. Chief Product Testing, DTS/CBER

Paul Mied, Deputy Chief Retrovirology, DTS/CBER

Jay Epstein, Chief Retrovirology, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Food and Drug Administration (FDA) regulates the manufacture of test kits for the detection of HIV-1, HIV-2 and HTLV-I infection. As part of the regulation of these tests the FDA requires every lot of kits to pass a lot release test. In this study we have analyzed HTLV-I lot release data to determine the lot-to-lot variability of the kits over time and to determine the characteristics of specimens that make up a "good" panel member. The kits monitored were from Abbott Labs, Cambridge Biotech, Cellular Products, and Organon Teknika. The lot release panel used was panel 2F which contained 8 required positive, 2 not required positive and 2 negative specimens.

All kits submitted for lot release passed the release criteria. The sensitivity of kits varied in their ability to detect the "not required" panel members. The Organon kit routinely detected 10 of 10, Abbott 9 of 10, and Cambridge and Cellular Products 8 of 10. The Coefficient of Variation (C.V.) for the kit cutoff factor ranged from 6 to 28%, while the C.V.'s for individual panel members ranged from 11 to 43%. Trend analysis (linear regression) of all panel members found no significant changes in kit sensitivity (slope was not significantly different than zero) during the study period.

These studies allow for the preparation of a new lot release panel based upon the lower limit of variation for each test. In addition, the defining of kit variation allows for more critical monitoring of changes in kit sensitivity. These analytical methods can be easily applied to lot release panels for HIV-1 and HIV-2 antibody detection tests to improve the monitoring of test kit consistency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06061-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of a lot release serum panel for HIV-2 antibody detection tests

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald E. Mayner, Chief Immunochemistry, DTS/CBER

Other: Mouna P. Selvam, Staff Fellow, DTS/CBER

Paul Mied, Deputy Chief Retrovirology, DTS/CBER

Jay Epstein, Chief Retrovirology, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Food and Drug Administration (FDA) regulates the manufacture of test kits for the detection of HIV-2 infection. As part of the regulation of these tests the FDA requires every lot of kits to pass a lot release test. In this study we have used commercial and in house tests to characterize the antibody specificities of prospective sera for use as lot release panel members. Twenty sera were obtained for analysis.

Based upon commercial viral lysate tests these sera were categorized into two groups (predominately HIV-2 and dual HIV-1, HIV-2 reactive). All specimens were characterized as having strong gag and env reactivity on HIV-2 RIPA and western blot (WB) assays. Predominantly HIV-2 reactive specimens were characterized as having strong reactivity to env peptides on HIV-2 WB and weak reactivity to gag and env peptides on HIV-1 WB. Dually reactive specimens, in general, gave only slightly stronger gag and env reactivity on HIV-2 WB than HIV-1 WB. These data suggest that reactivity with gag epitopes are primarily responsible for cross reactivity of HIV-2 infected individuals reacting with HIV-1 antigens.

Further studies using synthetic peptides with these sera should help to further discriminate between cross reactivity and the possibility of dual infections by identifying the reactive epitopes with genetically conserved epitopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06062-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of monoclonals to HTLV II

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Martin Ruta, Ph.D.

Other: Gao Liu, Fogarty Fellow

DTS/CBER

Roy Blay, Ph.D.

DTS/CBER

Jay Epstein, M.D. Lab Chief

DTS/CBER

Ron Mayner, Section Chief

DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Retrovirology

SECTION

HTLV

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We are generating monoclonal antibodies to HTLV II viral proteins for use in Western blot and RIPA analysis of HTLV II infected cells. Mice were immunized with detergent disrupted HTLV II. Following repeated boosts, the mice were bled and the polyclonal sera tested on HTLV I and HTLV II Western Blot for reactivity. The mice were sacrificed and spleen cells fused with a fusion partner to generate hybridoma cell lines. 26 cell lines were cloned and chosen for study based on the reactivity of the cell supernatant on HTLV I and HTLV II ELISA assays. Preliminary results show reactivity of supernatant from 8 clones with a band that appears to be the HTLV II envelope glycoprotein on Western blot. The supernatants will be further characterized to determine their reactivities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06063-01(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Two-in-one PCR for HIV-1 and HIV-2.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : Udaykumar , Fogarty Fellow DTS/CBER Others : Indira K. Hewlett, Section Chief DTS/CBER Alonso Heredia, Fogarty Fellow DTS/CBER Jay S. Epstein, Lab. Chief DTS/CBER		
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Retrovirology</p>		
SECTION <p style="text-align: center;">HIV Molecular Biology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">DTS/CBER/FDA</p>		
TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.20	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> PCR is a powerful technique that has been used widely in the diagnosis of HIV infections. PCR based diagnostic techniques have primarily focused on HIV-1 as it is the type prevalent in the US, the western hemisphere and most of Africa. However, AIDS cases due to HIV-2 infection are consistently on the rise, especially in West Africa and many less developed countries, making it necessary to develop PCR assays to detect HIV-2 strains. There have been several PCR assays designed to fulfill this requirement. As all the PCRs reported to date are specific either for HIV-1 or HIV-2, a clinical evaluation in a population with significant numbers of infections due to HIV-1, HIV-2 or both viruses would require the use of both assays. In order to circumvent this double test approach, we designed PCRs so that a single assay would be able to differentiate between both viruses by giving different amplification signals. We selected gag, pol and LTR regions for this purpose and presently are in the process of optimizing the assays. These primers will be used in clinical evaluation studies on samples collected from different geographical regions. They will also be used in viral infection studies as well. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06064-01(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) In situ PCR for detection of HIV-1 and HIV-2		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Bharat Joshi DTS/CBER Other: Indira K. Hewlett DTS/CBER Uday Muppala DTS/CBER Gary Riordan DTS/CBER Jay S. Epstein DTS/CBER		
COOPERATING UNITS (if any) Basil Golding, DH/CBER		
LAB/BRANCH Lab. of Retrovirology		
SECTION Molecular Biology		
INSTITUTE AND LOCATION CBER/FDA		
TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.20	OTHER: 0.05
CHECK APPROPRIATE BOX(ES): <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.) <p> We are currently setting up a technique that will detect HIV infected cells in a population of uninfected ones in the absence of detergent or phenol treatment using In Situ Polymerase Chain Reaction. The rationale of the technique is to set up a sensitive and reproducible test that will screen out infected cells non-isotopically by visual examination under an optical microscope. The technique will fix the cells onto the wells on a teflon coated slide. The cells will then be washed and made permeable with a quick proteinase K digestion. They are then reacted with PCR reaction mixture containing SK38/39 primers for 35 cycles at 91, 55, and 65 °C after covering with coverslips. The amplified products within the cells can be hybridized with biotinylated SK 19 probe and reacted with a streptavidin- peroxidase system for color development. The positive cells stain brownish-red and can be scored visually under optical microscope. At the present time, several methods of fixation and several fluorophores for labelling of primers and probes are being explored. Methods for detection of amplified material include analysis by flow cytometry. </p> <p> We will use the technique in detection of HIV virus in different physiologic materials, in assessing the effectiveness of anti-viral drugs against HIV and in understanding the role of host and viral factors in pathogenesis of the disease. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06065-01 (LR)

PERIOD COVERED

October 1, 1991 to September 1, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anti-HIV activity of antineoplastons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira Hewlett, Section Chief, DTS/CBER

Other: Sherwin Lee, Bio. Lab Tech, DTS/CBER

Jay S. Epstein, Lab Chief, DTS/CBER

COOPERATING UNITS (if any)

Dr. Burzinski, Burzinski Institute, Houston, Texas

LAB/BRANCH

Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antineoplastons are peptides and amino acid derivatives isolated from urine and known for their antineoplastic effects in various cancers. These agents are currently being administered to HIV infected individuals for treatment of their lymphomas. In the course of treatment it was noticed that the HIV antigen levels decreased. It was therefore of interest to assess the anti-HIV effects of these agents. Three different antineoplastons, sodium phenyl acetylglutamate (PG) sodium phenyl acetate (PN) and a mixture of PG and PN in the ratio of 1:4 were tested against HIV in target H9 and U937 cells. The concentrations used were 0.5, 0.1, 0.05, and 0.025 ug/ml. Preliminary results suggest that PN and PG at 0.05 and 0.1 ug/ml inhibit HIV replication by greater than 50% with minimal toxicity. We are at present evaluating other concentrations of these drugs for their effect on HIV replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06066-01(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Persistent HIV-1 infection of human mammary epithelial cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Kimber L. Poffenberger, Sr. Staff Fellow DTS/CBER OTHER: Indira K. Hewlett, Section Chief DTS/CBER Gary S. Riordan, Bio. Lab. Tech. DTS/CBER Sherwin Lee, Bio. Lab. Tech. DTS/CBER Jay S. Epstein, Lab. Chief DTS/CBER		
COOPERATING UNITS (if any) Lab. of Cell Biology, DH		
LAB/BRANCH Retrovirology		
SECTION Molecular Biology		
INSTITUTE AND LOCATION DTS/CBER/FDA		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We are studying cell lines isolated from both normal breast and breast carcinomas to determine whether mammary epithelial cells (MEs) can be infected by HIV-1. MEs are a potential reservoir for HIV and may be a source of the HIV detected in the breast milk of seropositive mothers. HIV-1 (MN) was used to infect six different ME cell lines (as well as H9 cells for comparison), which were monitored for virus production by p24 antigen assay, DNA PCR analysis and immunofluorescence. These infected cultures have been maintained for over 60 days and are continuing in culture. Low level infection is detectable by p24 assay and by DNA PCR. The time course of infection as measured by p24 antigen varied among different cell lines. Experiments are in progress to 1) ascertain whether these cells are producing infectious virus and to 2) identify the cells and/or cellular factors which may be involved in maintaining a latent HIV infection. </p> <p> This work has been published as an abstract in the 1992 Keystone Symposia on Prevention of AIDS. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06067-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

False positive reactions on donor screening tests and correlation with vaccination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul A. Mied, Ph.D., Deputy Chief, Laboratory of Retrovirology
Other: Jay S. Epstein, M.D., Chief, Laboratory of Retrovirology
Charles O. Roberts, Ph.D., Laboratory of Retrovirology
Leonard Wilson, Laboratory of Hepatitis

COOPERATING UNITS (if any)

Laboratory of Respiratory Virus Diseases, Division of Virology

LAB/BRANCH

Retrovirology

SECTION

Retrovirology Office

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The phenomenon of multiple false positive reactions on donor screening tests for HIV-1, HTLV-I, and HCV, and a possible correlation with flu vaccination was first described by kit manufacturers in December, 1991. We obtained aliquots of 64 donor samples which exhibited false positivity in screening tests in the fall of '91. A survey of licensed test kits indicated that the kits of several manufacturers were involved. False positive reactions with these samples ranged from 0-100% in HIV kits, 2-86% in HTLV kits, and 0-63% in HCV kits. A single anti-HBc kit tested was falsely positive for 23% of the samples. A panel was prepared of pre- and post-vaccination sera from 156 individuals who received the flu vaccine either in 1990-91 or 1991-92. False positive reactions following influenza vaccination were detected with a single HIV test kit at a rate which exceeded that found in clinical surveillance studies. Two panels were also prepared of pre- and post-vaccination sera from 100 individuals each who received hepatitis B vaccine from one of two different manufacturers. For one of the two panels, false positive reactions following hepatitis B vaccination were detected with a single HTLV test kit in 58% of the vaccinees, suggesting that at least some of the cases of false positivity may be correlated with vaccination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06068-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of antisense immunoliposomes on HIV-infected H9 cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Roy A. Blay, Ph.D., Senior Staff Fellow, DTS/CBER

Sheila M. Buck, Laboratory Tech., DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research demonstrates the sequence specific suppression of HIV replication using HIV-1 antibody-targeted liposomes containing antisense phosphorothioate oligonucleotides. Liposomes were generated which encapsulated the 20-mer sequence of the rev HIV-1 regulatory gene in the form of a phosphorothioate oligonucleotide. Specific targeting of the liposome was accomplished by conjugating HIV positive human IgG to the surface of the liposome resulting in the formation of an immunoliposome. HIV-1 infected H9 cells incubated with the immunoliposomes were rendered less permissive for viral replication. As compared with the positive control, HIV replication was reduced by almost 95%. Inhibition of HIV replication was not observed using empty liposomes containing random phosphorothioate oligomer sequences. These immunoliposomes exhibit dual specificity: a targeting antibody on the surface of the liposome specific for infected cells, and, inside the immunoliposomes, an oligomer with antiviral activity that is complementary to a specific portion of the mRNA of the infected cell. The antiviral activity of the free and the encapsulated oligonucleotides was assessed by p24 antigen ELISA, reverse transcriptase assay, Western blot, immunofluorescence, and PCR analysis. Liposome preparations demonstrated minimal toxicity in H9 cell culture experiments. Results using targeted antisense liposomes suggest that the mechanism for the inhibition of viral expression is its interaction with the rev regulatory gene resulting in translation arrest. These in vitro culture results demonstrate the potential efficacy of drug-encapsulated immunoliposomes in the treatment of AIDS and AIDS related complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06069-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibition of transformed human mammary epithelial cells by antisense oligos

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

COOPERATING UNITS (if any)

David S. Salomon, Ph.D., Section Chief, LTIB/NCI
Nicola Normanno, M.D., Fogarty Fellow, LTIB/NCI

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Amphiregulin (AR) is a member of the epidermal growth factor (EGF)/transforming growth factor α (TGF α) gene family since it structurally resembles EGF or TGF α , and it can bind to the EGF receptor. MCF10A cells are a spontaneously immortalized, nontransformed human mammary epithelial cell line that requires EGF for growth. AR (0.05-0.5nM) was found to stimulate the growth of MCF10A cells to a level that was comparable to the growth stimulation produced by equivalent concentrations of EGF. MCF10A cells express low levels of a 1.4kb mRNA transcript for AR. MCF10A cells transformed by either an activated human c-Ha-ras protooncogene or with a nonactivated overexpressed rat c-neu (erb B-2) gene exhibited a 15-30 fold increase in the level of AR mRNA expression. Endogenous AR protein could be immunocytochemically detected in MCF10A cells using a polyclonal antibody obtained after immunizing rabbits with a 19-mer peptide corresponding to the NH2 terminus of AR. MCF10A parental cells exhibited a weak cytoplasmic staining with this antibody whereas c-Ha-ras or c-erb B-2 transformed MCF10A cells differ from the pattern of endogenous TGF α expression which was enhanced in c-Ha-ras but not in c-erb B-2 transformed MCF10A cells. In order to ascertain what role AR might perform in MCF10A transformed cells, we synthesized two different phosphorothioate 20-mer antisense oligodeoxynucleotides complementary to the 5' sequence of AR mRNA starting respectively 3 and 9 nucleotides upstream of the AUG translation initiation codon. These antisense oligodeoxynucleotides and a 20-mer missense oligonucleotide at a concentration of 10 μ M were able to inhibit the soft agar growth of c-Ha-ras and c-erb B-2 transformed cells by 70-80% as compared to the control missense treated cells. These data suggest that AR may function as an autocrine growth factor in mammary epithelial cells transformed by ras and erb B-2 oncogenes and that these oncogenes can differentially modulate the expression of various members of the EGF family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 BG06070-01(LR)
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Detection of HIV by a magnetic bead based viral capture assay		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Indira K. Hewlett Section Chief DTS/CBER Other: Bharat Joshi Visiting Fellow DTS/CBER Gary Riordan Bio. Lab. Tech DTS/CBER Jay S. Epstein Lab. Chief DTS/CBER		
COOPERATING UNITS (if any)		
LAB/BRANCH Lab. of Retrovirology		
SECTION Molecular Biology		
INSTITUTE AND LOCATION CBER/FDA		
TOTAL STAFF YEARS: 0.75	PROFESSIONAL: 0.6	OTHER: 0.15
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A novel technique is being developed for detecting HIV from body fluids such as serum, plasma and culture supernatants of infected cells. H9 cells were infected with HIV-MN strain and the culture supernatants collected on day 4. p24 antigen was measured in the supernatant. Virus particles present in the fluids were concentrated by capture with antibody coated beads. Particles of uniform size were coated primarily with anti IgG antibodies and then reacted with anti p24 and anti gp120 separately, both being IgG class antibodies. After incubation for 16 hours in the cold, the beads were washed with buffer and stored at 4°C. Culture supernatants were then incubated with the beads in the cold for 16 hours. The beads were washed twice and treated with guanidine isothiocyanate buffer to strip off the virus. RNA from the stripped material was prepared and used for RT-PCR using SK 38/ 39 primers and amplified products were hybridized with P-32 labelled SK 19 probe by liquid hybridization technique. In our preliminary experiments, virions were enriched with magnetic beads coated with anti HIV antibodies and showed strong positive signals on an autoradiographic blot. This method is being adapted to detecting virus in the serum and plasma of infected individuals in an effort to derive sensitivity and specificity. This technique will be of use in detecting virus as well as in characterizing virus isolates by PCR sequencing directly from the bead.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BGD6071-01(LR)

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

HIV survival in packed red cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. J. Geyer Senior Staff Fellow DTS/CBER

Others: R. Blay Senior Staff Fellow DTS/CBER
N. Neiger Biologist DTS/CBER
J. S. Epstein Laboratory Chief DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

In FY91, DTS developed an urgent need to evaluate the safety of stored packed red cells (prbc's) with regard to transmission of bacterial and viral agents. Concern about the risk of sepsis and death due to transfusion of yersinia-contaminated prbc's prompted the Blood Products Advisory Committee to recommend reducing the storage time for packed cells. Available data suggested that deaths associated with yersinia contaminated units tend to increase with the time cells are stored at 2-6°C. In contrast, evidence suggests that the risk of transmission of HIV by transfusion of contaminated units decreases with longer blood storage time.

A novel infectivity assay developed under project Z01 BG 0631-02(LR) was used to experimentally determine the survival of cell-free and cell-associated HIV-1 virus in prbc's stored at 2-6°C in commonly used anticoagulants, CPDA and ADSOL. Donor prbc's collected in ADSOL or CPDA were spiked with cell-free and cell-associated HIV-1 virus and stored in collection bags at 2-6°C. Samples were drawn and exponential decline of infectious HIV-1 with time was determined. In duplicate experiments, the half-life of infectious HIV-1 was shown to be 3.0 days when stored in prbc's in CPDA and 7.5 and 4.2 days when stored in prbc's in ADSOL. The data was presented at the annual meeting of the American Association of Blood Banks in Baltimore, November 9-14, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06072-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoliposomal Adriamycin as an antiviral agent in HIV-infected H9 cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Sheila M. Buck, Laboratory Tech., DTS/CBER

Roy A. Blay, Ph.D., Senior Staff Fellow DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project will evaluate the anti-HIV activity of adriamycin (doxorubicin), an anticancer drug which inhibits some viral reverse transcriptases (RT) in H9 cells infected with HIV-1 or HIV-2. HIV infects not only T cells but also cells of the mononuclear phagocytic system. We are developing a system in our laboratory to test antiviral drugs against HIV infected cells. Doxorubicin has been shown to be efficacious in the treatment of infectious Kaposi's sarcoma. Preliminary experiments demonstrate that treatment with 20-100 nanograms/ml of doxorubicin effectively inhibits HIV replication in H9 cells, but, at the same time, it is more toxic to the cells. To reduce the toxicity and make delivery of the drug to the target more specific, immunoliposomes are being prepared using HIV antigen-specific IgG conjugated to the liposome to specifically target infected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06073-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

HTLV II ELISA and Western Blots

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Martin Ruta, Ph.D.

Other: Gao Liu, Fogarty Fellow

Jay Epstein, M.D. Lab Chief

Ron Mayner, Section Chief Immunochemistry

COOPERATING UNITS (If any)

LAB/BRANCH

Retrovirology

SECTION

HTLV

INSTITUTE AND LOCATION

DTS/CBER/FDA

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

OTHER:

0.3

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We have generated an ELISA for HTLV II antibody based on whole viral lysate. Purified HTLV II virus was detergent disrupted and used to coat 96 well plates. Optimum protein coating levels were determined. We are currently optimizing the conditions for testing HTLV I/II positive serum samples. Our results indicate that the HTLV II ELISA showed good specificity and sensitivity on samples that were confirmed as HTLV I or II positive by PCR.

We have also generated an HTLV II Western blot using detergent disrupted HTLV II viral antigen. We have analyzed the differential ability of HTLV I and HTLV II blots to detect antibodies from patients infected with HTLV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06074-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of antibody-complement mediated lysis of HIV-2 infected cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

Ronald E. Mayner, Section Chief, DTS/CBER

Sheila M. Buck, Laboratory Tech., DTS/CBER

Jay S. Epstein, M.D., Laboratory Chief, DTS/CBER

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.05

OTHER:

0.05

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Anti-HIV-1 positive antisera were tested for their ability to lyse HIV-1 infected cells in the presence of complement. We will test a panel of anti- HIV-2 positive human sera for their ability to activate human complement by the classical pathway using HIV-2 infected cells. Activation will be assessed by the flow cytometric detection of cell surface C3 deposition using C3-IgG conjugated to FITC. Analysis of complement-mediated cytolysis of infected cells using defined antisera against recombinant HIV-1 env or core antigens suggested that the env gp160/120 and p24 (gag) act as target antigens for antibody and complement mediated cytolysis. Cooperative effects of specific antibodies and complement inhibited HIV infection. We will compare the relative efficiencies of human positive sera and monospecific rabbit sera for their roles in complement mediated lysis of HIV-2 infected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06075-01(LR)

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amphiregulin as an autocrine growth factor for human colon carcinoma cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mouna P. Selvam, Ph.D., Senior Staff Fellow, DTS/CBER

COOPERATING UNITS (if any)

David S. Salomon, Ph.D., Section Chief, LTIB/NCI
Nicola Normanno, M.D., Fogarty Fellow, LTIB/NCI

LAB/BRANCH

Laboratory of Retrovirology

SECTION

Immunochemistry

INSTITUTE AND LOCATION

CBER, FDA

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Amphiregulin (AR) is a secreted heparin binding growth factor that is structurally and functionally related to epidermal growth factor (EGF) and transforming growth factor α (TGF α). GEO and WIDR cells are human colon cancer cell lines. Northern blot analysis of poly (A) mRNA showed that GEO cells but not WIDR cells express a 1.4kb specific AR transcript. However, endogenous AR protein could be immunocytochemically detected in both cell lines, with GEO exhibiting a higher level of staining than WIDR cells. Two different phosphorothioate 20-mer antisense oligonucleotides complementary to the 5' sequence of AR mRNA were able to inhibit the growth of GEO and WIDR cells. The two antisense oligonucleotides at a concentration of 10 μ M inhibited the soft agar growth of GEO and WIDR cells by 90% and 50%, respectively, as compared to the untreated control cells. A missense oligonucleotide had no effect on the growth of the two cell lines. An inhibition of 50% of monolayer growth of GEO cells treated with the same concentration of antisense was observed. These data suggest that AR may play a role as an autocrine growth factor for human colon carcinoma cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06076-01(LR)

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Viral etiology of AIDS associated KS in HIV negative individuals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira K. Hewlett

DTS/CBER

Other: Sherwin Lee

Bio. Lab. Tech

DTS/CBER

Bharat Joshi

Visiting Fellow

DTS/CBER

Jay S. Epstein

Lab. Chief

DTS/CBER

COOPERATING UNITS (if any)

Vincent Soriano

Barcelona Hospital, Madrid, Spain

LAB/BRANCH

Lab. of Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The HIV status of a 42 year old homosexual male, who was seronegative, but had symptoms of Kaposi's Sarcoma was determined by PCR. This individual was PCR -ve and Ab -ve. RNA-PCR on peripheral blood lymphocytes using tat primers also yielded negative results, suggesting that this may be a case of KS in the absence of HIV infection. A second case was evaluated where a patient with low CD4 counts and disseminated tuberculosis was negative for HIV by PCR and serologic tests. At present experiments are in progress to study the role of other viruses in Kaposi's Sarcoma in patients with immune deficiency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BG06077-01(LR)

PERIOD COVERED

October 1, 1991 to September 1, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Evaluation of silent HIV infection in high risk individuals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Indira K. Hewlett, Section Chief DTS/CBER

Other: Sherwin Lee, Bio. Lab. Tech DTS/CBER

Jay S. Epstein, Lab. Chief DTS/CBER

COOPERATING UNITS (if any)

Stanley Weiss, Univ. of Medicine and Dentistry of NJ
Gene Shearer, NIAID, NIH

LAB/BRANCH

Lab. of Retrovirology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

CBER/FDA

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.20

OTHER:

0.05

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The prevalence of silent HIV infection in a cohort of individuals at risk for HIV infection (IVDUs and spouses) is being analyzed by PCR and a cell proliferation assay based on the release of cytokines in the early phase of infection with the virus. PCR is being performed on DNA isolated from peripheral blood mononuclear cells and on RNA from serum. The primers used for amplification are the SK 38/39 and SK 145/431. Preliminary results indicate that there is a low but definite incidence of HIV infection in these high risk individuals (3/37 positive by PCR). All of these individuals have been negative for RNA in serum analyzed by PCR.

APPENDICES 1-6

1. Blood Products Advisory Committee Agenda Items.
2. Guidances to Blood Establishments
3. Committee Liaisons
4. Memorandum concerning Regulatory issues and Future needs.
5. LBBP, Standard Operating Procedures
6. Draft of Quality Assurance Guidance

FY92 Blood Products Advisory Committee MeetingsDecember 12-13, 1991

Meeting 34

OPEN

1. Review of Hemophilia and Inhibitor Problems
2. Overview of Hemophilia and Inhibitor Problems
3. Open Public Hearing

CLOSED

Presentation by manufacturer

4. Review of application for Recombinant Factor VIII-Miles, Inc.
5. Review of application for Recombinant Factor VIII-Baxter Laboratories
6. Review of scientific laboratory site visits
 - a. Laboratory of Retrovirology-DTS
 - b. Laboratory of Cellular Hematology-DH

CLOSED

Committee discussion

March 12-13, 1992

Meeting 35

OPEN

March 12, 1992

1. Recommendation for use of new multi-antigen screening tests that detect antibodies to the Hepatitis C virus
2. Issues related to use of the Chiron RIBA - II[™] immunoblot assay for antibody to HCV
3. Re-entry of donors with repeatedly reactive screening test for anti-HCV
4. Review and discussion of false positive screening test results associated with influenza immunization

March 13, 1992

1. Advisory Committee consideration of FDA recommendations pertaining to blood issues
 - A. HIV-related donor deferral criteria
 - B. Discussion of "fresh" blood requirements and laboratory testing procedures
2. Presentations
3. FDA Questions
4. Discussion and Recommendations

May 28-29, 1992

Meeting 36

Open

May 28, 1992

Invalidation of test results when screening donor blood using licensed Viral Marker Test Kits

May 29, 1992

Review of the problem of bacterial contamination of platelets.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20892

Date: December 12, 1991

From: Acting Director, Center for Biologics Evaluation and Research

Subject: Clarification of FDA Recommendations for Donor Deferral and Product Distribution Based on the Results of Syphilis Testing

To: All Registered Blood Establishments

Background

For at least 30 years, a screening test for syphilis has been performed as part of routine laboratory testing on every unit of blood or blood component for transfusion. Most, if not all, states have required reporting of reactive screening results to the Department of Health, and confirmatory testing has usually been done by health departments rather than blood centers. Whole blood and red blood cells with reactive screening results and negative confirmatory results are usually discarded, although FDA has stated that use is acceptable if units are labeled with both the reactive screening test result of record and the negative confirmatory test. Source Plasma collected prior to receipt of screening test results has been considered acceptable for further manufacturing. Donors whose blood or plasma tested reactive were not in the past deferred for any specific time period, but were often asked to provide evidence of treatment before donating again.

FDA regulations require that Source Plasma donors be tested for syphilis on the day of first medical examination for plasmapheresis and at least every four months thereafter. The regulations do not require the labeling of each unit with the screening test results. FDA has not recommended product retrieval when repeat donors test reactive for syphilis because transmission of syphilis has not been considered a health risk for plasma derivatives. For continued Source Plasma collection from a donor who is known to have a reactive test for syphilis, documentation of medical treatment is required (21 CFR 640.65) (2) (iv).

In the memorandum to all blood establishments dated December 5, 1990, the Food and Drug Administration (FDA) recommended that blood establishments defer potential blood and plasma donors who provide positive responses to a question about having had or having been treated for syphilis or gonorrhea during the preceding 12 months. Since publication of this recommendation, a number of blood establishments have sought clarification as to whether this additional donor suitability criterion has altered

the way in which tests for syphilis should be used as a basis for donor deferral, removal of product from use channels, or recipient tracing. The purpose of this memorandum is to clarify FDA's recommendations on the use of tests for syphilis in regard to AIDS-related risk.

Rationale for Change

Asking donor history questions concerning risk factors and performing serological tests for markers of infectious diseases are each important elements to better assure the safety of the blood supply. In recommending that donors who have a history of diagnosis or treatment for syphilis or gonorrhea in the last 12 months be deferred, the FDA intended to broaden the donor deferral criteria to exclude additional individuals who might be at increased risk of HIV infection due to heterosexual transmission. Although recommendations for management of units or donors based on the results of serological testing for syphilis were not specifically addressed earlier, it cannot be ignored that a confirmed positive screening test for syphilis is in most cases evidence of recent or untreated syphilis and that this information can supplement the use of donor history questions in making deferral decisions.

Donor Deferral Recommendation

To ensure a uniform approach to these issues, FDA is now making an additional recommendation that may be implemented immediately. Concurrent with implementation, licensed establishments should revise their standard operating procedures and submit a statement to their license application file containing the information indicated in the attached example. This statement indicates that a revised standard operating procedure consistent with the following recommendation has been implemented:

Donors who are found to have a reactive screening test for syphilis by the Automated Reagin Test (ART), the Rapid Plasma Reagin Test (RPR), the Venereal Disease Research Laboratory Slide Technique (VDRL), or other screening test, should be temporarily deferred pending the outcome of a confirmatory test such as the Fluorescent Treponemal Antibody Absorption Test (FTA). Donors who are found to have a positive FTA (or other confirmatory test), or for whom no additional test result is available, should be deferred for 12 months. (In the event that a history of therapy for syphilis in the last 12 months is established, the 12 month deferral period may be calculated from the established date of diagnosis.) After 12 months, deferred donors may be requalified if they have a negative screening test. For cases in which the positive serology was

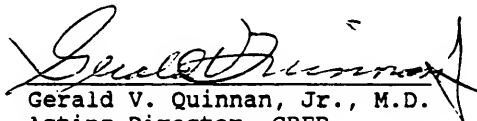
confirmed, evidence of adequate treatment for syphilis, documented by a letter from a physician or public health clinic, should also be obtained.

The following additional points of clarification are intended to resolve confusion which may exist regarding current recommendations:

1. FDA is not recommending at this time that extant units of whole blood, blood components, and Source Plasma be removed from use channels if previously collected from donors who subsequently provide a history of syphilis or gonorrhea within the last 12 months, or who are found subsequently to have reactive or confirmed tests for syphilis and have no other disqualifying history or test results, nor is FDA recommending that the blood center notify hospitals for the purpose of tracing recipients of such products.
2. For Source Plasma, current collection and labeling requirements related to the results of serologic tests are found in 21 CFR 640.65 and 21 CFR 640.70. FDA is not recommending that the frequency of serological testing for syphilis for Source Plasma be altered (i.e., initially, then every four months) and Source Plasma collected before serologic test results are received may be used for further manufacturing.
3. For whole blood and blood components, otherwise suitable units may be released for transfusion if they were obtained from donors who tested reactive for syphilis by screening tests but had negative results of confirmatory testing by FTA or equivalent methods on the same collection. For units to be released for transfusion, the confirmatory test should be performed on every donation for which there is a reactive screening test (i.e., prior negative confirmatory results are insufficient to qualify a collection with a reactive screening test). Such units should be labeled as reactive by a screening test for syphilis and negative by FTA (or other confirmatory test).
4. In cases of autologous donation of blood or components, collections with reactive screening tests which are either positive by FTA, or not further tested, may be used for transfusion provided that they bear both an "Autologous Use Only" label and a biohazard label. A written report of the test results should be provided to the patient's physician before transfusion. Guidance on proper labeling of autologous blood and components may be found in memoranda dated March 15, 1989 and February 12, 1990. Biohazard labels are described in the current Guideline for the

Uniform Labeling of Blood and Components.

It should be noted that the recommendation on labeling of autologous blood and components reactive for syphilis differs from FDA recommendations issued on March 15, 1989, with respect to restrictions on the use of autologous blood which is positive for other infectious disease markers. For release of autologous units found to be repeatedly reactive for anti-HIV or HBsAg, the physician's written request remains necessary.



Gerald V. Quinnan, Jr., M.D.
Acting Director, CBER

Gerald V. Quinnan, Jr., M.D.
Acting Director
Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
ATTN: HFB 240

Dear Dr. Quinnan:

In response to your recent recommendations for revision of STS positive donor deferral and product distribution procedures affirm that:

_____ Our program is in complete compliance with FDA's recent recommendations included in the memorandum to all registered blood establishments dated Dec. 5, 1990.

_____ A revised standard operating procedure consistent with the recommendations has been implemented.

Date implemented: _____

Please add this information to the license file for facility:

License No. _____

Registration No. _____

Facility Name _____

Address _____

Responsible Person: Name _____

Telephone No. _____

Date of response _____

Sincerely yours,

Signature



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20892

Date: 01/28/92

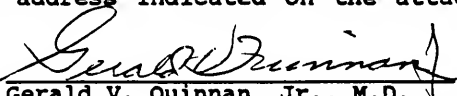
From: Acting Director, Center for Biologics Evaluation and Research

Subject: Notification to FDA of implementation of updated standard operating procedures on donor deferral for the prevention of Human Immunodeficiency Virus (HIV) transmission (follow-up to December 5, 1990 memorandum).

To: All Licensed Blood Establishments

On December 5, 1990, the Food and Drug Administration issued a revision and update of donor selection and deferral recommendations related to the prevention of HIV transmission by blood and blood products. The recommendations were made on the basis of new scientific information including data which were discussed at the FDA Blood Products Advisory Committee Meeting on April 20, 1990. These recommendations were designed to further reduce collections from potential donors at increased risk of HIV infection. The recommendations included enhanced donor educational efforts on risk factors through oral as well as written explanation and the inclusion of direct questions about AIDS-related high risk behavior. The recommendations also included a one year deferral for a history of diagnosis or treatment for syphilis or gonorrhea and extended to one year the deferral for transfusion recipients or individuals with a history of sex with a prostitute. The December 5 memorandum also recommended the discontinuance of the deferral related to emigration from sub-Saharan Africa for centers which test routinely for anti-HIV-2. Blood establishments were requested to implement the recommendations immediately and licensed establishments were requested to submit concurrently a statement to their files indicating that standard operating procedures consistent with the recommendations had been implemented.

Subsequent to issuance of these recommendations pursuant to 21 CFR 600.14, FDA has received an increased number of accident reports. These accident reports include the release of blood for distribution in which a donor subsequently acknowledges, either by telephone call or at subsequent donation, a basis for exclusion from blood donation. The reasons for these increased reports are unclear, but increased effectiveness of donor deferral screening may be a contributing factor. The FDA is attempting to further evaluate the temporal relationship between changes in deferral practices, subsequent deferrals and donor quality. For this purpose, we request that you complete the attached information request and return it to the address indicated on the attached document.


Gerald V. Quinnan, Jr., M.D.
Acting Director, CBER

Responding License No. _____
Establishment: Name _____
Address _____

Responsible Head: Name _____
Telephone No. _____
Fax No. _____
Signature _____
Date _____

1. Please check one response and provide additional information as indicated:

_____ Revised standard operating procedures consistent with all of the recommendations of the December 5, 1990 Memorandum have been implemented as of the following date: _____/_____/_____.

_____ We have not implemented all of the December 5, 1990 recommendations, or have implemented them in a manner inconsistent with that described in the memorandum. (Please attach an explanation including validation of the procedures implemented.)

2. Please provide the indicated information for the three months prior to and the three months subsequent to implementation of the December 5, 1990 recommendations:

<u>Item of Information</u>	<u>(Time Period Related to Implementation of December 5, 1990 Recommendations)</u>	
	<u>3 Months Prior</u>	<u>3 Months Subsequent</u>

No. repeat donors disqualified subsequent to donation due to information obtained on risk factors for HIV

Rate (No. per 10,000) of confirmed HIV-1 positives (EIA+/WB+) in screened donors

This form should be mailed to:

Gerald V. Quinnan, Jr., M.D.
Acting Director
Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike, HFB 240
Bethesda, MD. 20892

FY92 DTS INITIATED GUIDANCE TO BLOOD ESTABLISHMENTS

MEMORANDA FINAL:

1. Clarification of FDA Recommendations for Donor Deferral and Product Distribution Based on the Results of Syphilis Testing. To all Registered Establishments 12/12/91.
2. Notification to FDA of implementation of updated standard operating procedures on donor deferral for the prevention of Human Immunodeficiency Virus (HIV) transmission (follow-up to December 5, 1990 memorandum). To all Licensed Establishments 1/28/92.
3. Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV). To all Registered Establishments 4/23/92.
4. Use of Fluorognost HIV-1 Immunofluorescent Assay (IFA). To all Registered Blood and Plasma Establishments 4/23/92.
5. Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products. To all Registered Establishments 4/23/92.
6. Exemptions to Permit Persons with a History of Viral Hepatitis Before the Age of Eleven Years to Serve as Donors of Whole Blood and Plasma: Alternative Procedures, 21 CFR 640.120. To all Registered Establishments 4/23/92.
7. Changes in Equipment for Processing Blood Donor Samples. To all Licensed Establishments 7/21/92.

MEMORANDA IN PREPARATION

1. Revision of October 7, 1988 Memorandum Concerning Red Blood Cell Immunization Programs. To all Licensed Establishments Performing RED Blood Cell Immunization.
2. Nomenclature for Monoclonal Blood Grouping Reagents. To US Licensed Manufacturers of Blood Grouping Reagents.
3. Outside Testing Laboratories. To all Registered Establishments.
4. Volume Limits for Automated Collection of Source Plasma. To all Registered Establishments.
5. Hemapheresis Red Blood Cell Loss and Donor Deferral. To all Registered Establishments.
6. Identification of Problem Areas and Recommendations for Retrospective Validation of Computer Systems used for Tracking and Manipulation of Information Concerning Donors and Blood Products. To all Registered Establishments.
7. Recommendations for the Collection, Testing and Release of Autologous Blood and Blood Components. To all Registered Establishments.
8. Manufacturing Procedures for Irradiated Blood Products. To all Registered Establishments.

FEDERAL REGISTER NOTICE. Vol 57, No. 85, May 1, 1992, p. 18884-18887, concerning:

1. Points to Consider in the Manufacture of In Vitro Monoclonal Antibody Products for Further Manufacturing into Blood Grouping Reagent and Anti-Human Globulin, March 1992, Docket No. 91N-0466.
2. Recommended Methods for Blood Grouping Reagent Evaluation, March 1992 revision, Docket No. 84S-1081.
3. Recommended Methods for Anti-Human Globulin Evaluation, March 1992 revision, Docket No. 84S-1082.
4. Points to Consider in the Design and Implementation of Field Trials for Blood Grouping Reagent and Anti-Human Globulin, 1st draft 1992, Docket No. 91N-0467.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20892

Date: April 23, 1992

From: Director, Center for Biologics Evaluation and Research

Subject: Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV)

To: All Registered Blood Establishments

This memorandum transmits Recommendations for Testing for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV) in Blood Establishments, April 1992. These recommendations supersede those issued on 29 November 1990 which should be archived.

On 29 November 1990 the Food and Drug Administration (FDA) recommended that all Whole Blood and components for transfusion be screened to eliminate units that are repeatedly reactive for anti-HCV. At that time, the available licensed screening tests contained a single recombinant antigen for the detection of anti-HCV. Test kit manufacturers have recently developed kits capable of detecting antibodies to additional epitopes of the hepatitis C virus. In the memorandum of 29 November 1990, the FDA did not recommend withholding anti-HCV reactive plasma from further manufacture into plasma derivatives because the impact of this change on the safety of plasma derivatives such as immune globulin products had not been established. Information regarding the safety of such products has been collected since November 1990 by the FDA.

In a public meeting on 12 March 1992, after review and discussion of all the relevant information available, the FDA Blood Products Advisory Committee (BPAC) recommended that all donations of Whole Blood and blood components intended for transfusion, and Source Plasma and Source Leukocytes intended for further manufacture be screened for anti-HCV. The Committee also recommended that units repeatedly reactive for anti-HCV in multi-antigen tests should not be transfused. Accordingly, CBER is revising its recommendations.

FDA now recommends (1) that units of Whole Blood and blood components intended for transfusion, and Source Plasma and Source Leukocytes intended for further manufacture, be screened by an FDA licensed test for anti-HCV and (2) that no products repeatedly reactive for anti-HCV be used. The use of a multi-antigen test in the testing of Whole Blood and blood components for transfusion should be implemented as soon as is feasible. Initially reactive donor samples should be retested in duplicate

to determine whether they are nonreactive (negative) or repeatedly reactive. Only negative units are suitable for transfusion or for further manufacture, with the exception of autologous donations under specified conditions. Donors should be deferred indefinitely whenever they test repeatedly reactive for anti-HCV. A donor reentry protocol for anti-HCV cannot be recommended at this time because of the lack of an available licensed additional, more specific test.

Inventories of blood and blood components for transfusion that were collected before the multi-antigen test implementation date should also be tested using the multi-antigen test if possible. Inventories of products intended for further manufacture and collected before the test implementation date need not be tested retroactively.

The attached recommendations provide guidance on the testing, labeling, quarantine, storage and shipment of units of blood and blood components with respect to anti-HCV testing. Labeling, informed consent forms, standard operating procedures, deferral registries and recordkeeping procedures should be revised as necessary to reflect the blood establishment's implementation of anti-HCV testing.

Licensed manufacturers of Whole Blood and blood components may adopt labeling consistent with this Memorandum concurrently with submitting any changes in labeling (Section II) to the Division of Product Certification. Licensed manufacturers of Source Plasma should submit, within 90 days after receipt of this document, revised labeling for review and approval (Section III.A.). Approved labeling changes should be implemented within 180 days.

Section III.B. contains labeling recommendations of special interest to those involved in supplying Source Plasma, recovered plasma and red blood cells for research and for further manufacturing into noninjectable products. The labeling recommendations in Sections II and III should be implemented within 180 days after the receipt of this document.

Questions concerning testing may be directed in writing to the Food and Drug Administration, Division of Transfusion Science, Laboratory of Hepatitis, HFB-930, 8800 Rockville Pike, Bethesda, MD 20892, FAX: (301) 227-6764. Questions concerning labeling may be directed in writing to the Food and Drug Administration, Division of Product Certification, HFB-240, 8800 Rockville Pike, Bethesda, MD 20892, FAX: (301) 295-8528.



Kathryn C. Zoon, Ph.D.

Recommendations for Testing
for
Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV)
in
Blood Establishments

April 1992

U.S. Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research
8800 Rockville Pike, Bethesda, MD 20892

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IV. Quarantine and Disposition of Repeatedly Reactive Donations.....	8
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**RECOMMENDATIONS FOR TESTING FOR ANTIBODY
TO HEPATITIS C VIRUS ENCODED ANTIGEN
(ANTI-HCV) IN BLOOD ESTABLISHMENTS
April 1992**

I. PERFORMANCE OF ANTI-HCV TESTING AND DONOR SUITABILITY

- A. Anti-HCV testing, using multi-antigen tests, should be performed and test results interpreted according to the manufacturer's instructions in the package insert. Instructions for currently licensed kits may be summarized as follows:**
- 1. A single enzyme immunoassay (EIA) test for anti-HCV should be performed on a donor sample for each unit of whole blood or blood component intended for transfusion, and on each unit of plasma or leukocytes for further manufacture. This EIA will hereafter be referred to as the "initial test".**
 - 2. If the initial test result is nonreactive, the donor sample is considered to be negative for anti-HCV.**
 - 3. If the initial test result is reactive, the donor sample is considered to be initially reactive. The sample should be retested in duplicate, within a single run, using the same procedure and same manufacturer's test kit as that used for the initial test.**
 - a. If both duplicate repeat test results are nonreactive, the sample is considered to be negative for anti-HCV.**
 - b. If either one or both of the duplicate repeat test results are reactive, the test is considered to be repeatedly reactive for anti-HCV and the products should not be used for transfusion or for further manufacture. Possible exceptions to permit use of anti-HCV reactive products in special circumstances are described in Section I.C. and Section III.B. No further screening tests for anti-HCV should be performed on samples from this unit in an effort to qualify it as suitable.**
- B. Donors who are repeatedly reactive for anti-HCV, using multi-antigen tests, should be deferred indefinitely from donating blood and blood components for transfusion, Source Plasma and Source Leukocytes for**

further manufacture. General guidance in regard to the testing, counseling, and evaluation of donors tested for hepatitis viruses is described in the Public Health Service Interagency Guidelines for Screening Donors of Blood, Plasma, Organs, Tissues, and Semen for Evidence of Hepatitis B and Hepatitis C, MMWR 1991; 40 (RR-4): 1-17.

- C. No individual should be used as a source of Whole Blood, blood components, Source Plasma or Source Leukocytes as follows:
1. Individuals with a history of close contact within one year of donation with another individual having viral hepatitis.
 2. Individuals with a history of having received within one year human blood or any derivative of human blood which the FDA has advised the licensed establishment is a possible source of viral hepatitis (except in the case of specific immunization of Source Plasma donors [21 CFR 640.66]).

In addition it is recommended that individuals be deferred from donating Whole Blood, blood components, Source Plasma or Source Leukocytes, who within one year of donation have undergone acupuncture, ear piercing or tattooing in which sterile procedures were not used.

- D. Detailed guidance on the use of autologous units that are repeatedly reactive for anti-HCV was issued on 11 September 1991. FDA believes that public health considerations dictate the need for caution in the distribution and use of autologous products reactive for anti-HCV. Accordingly, repeatedly reactive units for autologous use should bear a restrictive label as recommended in Section II.B. Additionally, use of autologous blood that is repeatedly reactive for anti-HCV is acceptable provided that a report of the test result has been made available to the patient's physician. This recommendation differs from those issued on 15 March 1989 concerning the use of autologous blood positive for some other disease markers. The attending physician's written request is necessary for release of units that test reactive for anti-HIV and HBsAg, but is not required for release of autologous products reactive for anti-HCV.
- E. Test reactivity may represent a "false positive" reaction. In the absence of a licensed confirmatory test, it is suggested that an aliquot of serum or

plasma from each repeatedly reactive unit be frozen at -20°C or colder and stored for possible future use in verifying the screening test result in the context of a donor reentry algorithm.

- F. In the absence of a licensed confirmatory test for anti-HCV, the blood center may wish to utilize related test results when counseling the donor. The alanine aminotransferase (ALT) level of a donor sample may assist in the evaluation of the significance of a repeatedly reactive anti-HCV screening test result. However, regardless of the risk assessment from evaluation of other tests, donors who are repeatedly reactive for anti-HCV should be deferred indefinitely from donating whole blood and components for transfusion and plasma or leukocytes for further manufacture (See Section I.B.).

The American Blood Resources Association (ABRA) has prepared a guideline (Guideline for Anti-HCV screening in Plasmapheresis Facilities, Oct. 9, 1991) that may be referenced as an acceptable Standard Operating Procedure (S.O.P.) for the implementation of testing by manufacturers of Source Plasma.

- G. Informed consent forms, standard operating procedures and recordkeeping procedures should be revised as necessary to reflect the blood establishment's implementation of testing using FDA-licensed multi-antigen test kits.

II. LABELING OF WHOLE BLOOD AND COMPONENTS INTENDED FOR TRANSFUSION

- A. Whole Blood and Blood Components Intended for Allogeneic Transfusion

Consistent with the labeling for other infectious disease marker tests as described in 21 CFR 606.121 and in the current Draft Guideline for the Uniform Labeling of Blood and Blood Components, negative anti-HCV test results need not appear on the container label but should be included in the instruction circular. An appropriate statement is:

"...negative by a test for anti-HCV."

This statement may be combined with other statements concerning tests for infectious disease markers. For

example, the following combined statement is acceptable:

"A sample from each donation intended for homologous use has been tested by FDA-licensed tests and found negative for antibodies to human immunodeficiency virus (anti-HIV), hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis C virus (anti-HCV), and antibody to human T-cell lymphotropic virus, type I (anti-HTLV-I)."

B. Whole Blood and Blood Components Intended for Autologous Transfusion

The guidance for autologous blood and blood components issued on 15 March 1989 included recommendations for labeling autologous blood. When the test for anti-HCV is repeatedly reactive, the blood product should be permanently labeled with the special "FOR AUTOLOGOUS USE ONLY" and "BIOHAZARD" labels described in the current Guideline for the Uniform Labeling of Blood and Blood Components. The Circular of Information distributed with blood products should include an appropriate explanation concerning use of the biohazard label when an autologous unit tests repeatedly reactive for anti-HCV.

C. Units of Whole Blood or Components Not Tested for Anti-HCV or Not Tested for Anti-HCV Using a Multi-Antigen Assay.

On rare occasions, it may be necessary to ship a unit not tested for anti-HCV because a tested unit is not available. For example, untested Red Blood Cells, Frozen, with no serum or plasma available, may be required to meet an emergency need for a rare phenotype. The container label of such an untested product should include a statement such as the following:

"CAUTION: Test for anti-HCV has not been done".

If testing for anti-HCV has been performed using a single-antigen assay only, and not a multi-antigen assay, the container label should include a statement such as the following:

"CAUTION: Test for anti-HCV performed using a less sensitive test than one currently available".

III. LABELING OF BLOOD AND BLOOD COMPONENTS INTENDED FOR FURTHER MANUFACTURING OR RESEARCH

A. Plasma Intended for Further Manufacture into Injectable Products

Source Plasma and recovered plasma (shipped under short supply agreements) container labels should bear the statement, "Negative by a test for anti-HCV". This statement may be combined with the statement(s) regarding anti-HIV and HBsAg test results.

B. Plasma and Red Blood Cells Intended for Further Manufacture into Noninjectable Products; Whole Blood, Blood Components, and Samples for Research Use

Products intended for further manufacture into in vitro diagnostic reagents or for use in research studies are often provided to consignees on an "as needed" basis, rather than as routine shipments. Therefore, FDA is recommending that such products be labeled with one of the following statements to indicate test status:

1. "Negative by a test for anti-HCV."
2. "Not tested for anti-HCV."
3. "Reactive by a test for anti-HCV."

If the product is not tested for anti-HCV or is reactive for anti-HCV, labels should also include:

"CAUTION: For further manufacture only of in vitro diagnostic reagents for which there are no alternative sources."

or

"Not for use in products subject to license under Section 351 of the Public Health Service Act."

or

"For laboratory research use only."

The labeling statements addressed above pertain to units of blood or plasma collected from a donor that was not previously known to be anti-HCV reactive. The collection of Source Plasma from donors known to be anti-HCV reactive requires advance approval by the Director, CBER, of a specific product license application or amendment.

IV. QUARANTINE AND DISPOSITION OF REPEATEDLY REACTIVE DONATIONS

All donations that are repeatedly reactive for anti-HCV should be quarantined and either destroyed or restricted to appropriate use other than transfusion or manufacture into injectable products. Provisions of FDA's 6 April 1988 memorandum to all registered blood establishments, Control of Unsuitable Blood and Blood Components, apply.

- A. Whole Blood, blood components, Source Plasma and Source Leukocytes that have been found to be repeatedly reactive for anti-HCV should be moved from the general quarantine area for storage of untested units to a special quarantine area designated for units unsuitable for use due to infectious disease test results.
- B. Anti-HCV repeatedly reactive units should not be used for allogeneic transfusion or for further manufacture into injectable products. FDA suggests that establishments destroy the units and laboratory samples (except an aliquot of serum or plasma for future verification of donor status as noted in Section I.D.) by saturated steam autoclaving at 121.5°C maintained for 60 minutes, or by incineration. While awaiting destruction, Whole Blood and blood components, Source Plasma and Source Leukocytes should be quarantined and prominently labeled "NOT FOR TRANSFUSION; anti-HCV reactive" in accordance with 21 CFR 606.121 (f).
- C. Plasma, Whole Blood and blood components repeatedly reactive for anti-HCV should be distributed to consignees in a manner consistent with 21 CFR 606.40 (a)(6) and (7), and 606.100, 606.120, 606.121 and 606.165. Additional information concerning the shipment of biological products and clinical specimens, including donor blood samples, may be found in the following CFR sections:
 - 1. Postal Service: 39 CFR Part 111. See also (a) the Domestic Mail Manual, which is incorporated by reference into the CFR, and (b) the International Mail Manual, for materials to be transported by air.
 - 2. Department of Transportation: 49 CFR Part 173
 - 3. Department of Health and Human Services, Centers for Disease Control: 42 CFR Part 72
- D. The FDA's 26 October 1989 guideline (with a revision on 17 April 1991) for collection of blood or blood

products from "high risk" donors need not be applied at this time to collections from donors known to be anti-HCV repeatedly reactive, whose blood or plasma may be needed for autologous use, or for use in further manufacturing for in vitro products and in research (Section III). However, it is emphasized that all staff should be instructed to follow universal blood and body-fluid precautions in all situations where contact with human blood can be anticipated. (See: Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood-borne pathogens in health-care settings. MMWR 1988;37:377-386.)

V. "LOOKBACK"

A targeted "lookback" program in relation to previously collected products from donors testing repeatedly reactive for anti-HCV is not recommended at this time.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20892

DATE: April 23, 1992

FROM: Director, Center for Biologics Evaluation and Research

SUBJECT: Use of Fluorognost HIV-1 Immunofluorescent Assay (IFA)

TO: All Registered Blood and Plasma Establishments

On February 5, 1992, the Food and Drug Administration licensed Waldheim Pharmazeutika, GmbH, Vienna, Austria, to manufacture and distribute the Fluorognost HIV-1 IFA, an immunofluorescent assay (IFA) for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1) in human serum or plasma. The kit package insert states that the IFA test

"... is intended to be used as an additional, more specific test for antibodies to HIV-1 in human serum or plasma specimens found to be repeatably reactive by screening procedures, such as the Enzyme-Linked Immunosorbent Assay (EIA). Fluorognost HIV-1 IFA can also be used by properly trained personnel as a screening test in hospital laboratories, medical clinics, physicians' offices and emergency care situations and in blood banks or other settings where enzyme immunoassays are not practical or available."

The purpose of this memorandum is to provide further information on the approved use of this test in blood and plasma establishments.

The Fluorognost HIV-1 IFA uses infected, immortalized human T-cells that express antigens of HIV-1 (type III₂). The cells are fixed in wells on the surface of a glass slide. Separate wells of fixed, uninfected T-cells are provided as controls for reaction with the test sample. When antibodies to HIV-1 are present in a serum or plasma specimen, they will bind to the infected cells but should not bind to the control, uninfected cells. Bound antibodies are detected with anti-human immunoglobulin conjugated to fluorescein isothiocyanate. The conjugate becomes fixed to the bound human antibodies and emits light by fluorescence when exposed to UV light. The interpretation of the test result is based on a microscopic, visual evaluation of the degree and pattern of fluorescence of the infected cells compared with the uninfected cells. It requires approximately 1.5 hours to test one IFA slide containing five test wells.

As documented in the package insert, the Fluorognost HIV-1 IFA test, when properly performed, is equivalent in sensitivity to a licensed EIA for detection of antibodies to HIV-1 in human serum or plasma. Clinical studies have also shown that the Fluorognost HIV-1 IFA is equivalent in sensitivity and specificity to a

licensed Western blot for additional, more specific testing of EIA repeatably reactive serum or plasma samples obtained by screening in low or high risk populations. The data also suggest that the Fluorognost HIV-1 IFA may be useful in resolving the status of samples with an indeterminate Western blot result for purposes of medical counseling.

1. Use of the Fluorognost HIV-1 IFA as an additional, more specific test for validation of antibodies to HIV-1 in serum or plasma

The primary intended use of the Fluorognost HIV-1 IFA is as an additional, more specific test for the presence of HIV-1 antibodies in samples of human serum or plasma that are repeatably reactive by an EIA screening test. Therefore, the IFA can be used instead of an HIV-1 Western blot whenever an additional, more specific HIV-1 test is indicated. Such uses include testing for the purpose of donor notification and counseling, possible donor reentry, disposition of "lookback" product retrievals and "lookback" recipient notification. These uses are described below:

For purposes of donor notification, the licensed IFA may be used as an alternative to the HIV-1 Western blot. Also, if the IFA is performed subsequent to an indeterminate Western blot, the additional information from a negative IFA result may be used in donor counseling to alleviate anxiety over a test result that is unlikely to indicate HIV infection. Conversely, a positive IFA result would demonstrate the presence of antibodies to HIV, and the donor could be counseled accordingly.

When used to qualify a donor for reentry, the licensed IFA can be performed instead of the Western blot in any part of the recommended reentry algorithm (see Memorandum to All Registered Blood Establishments, February 5, 1990). Positive, negative and indeterminate results by the IFA should be treated the same way as the corresponding results of a licensed HIV-1 Western blot. Unlike the use in donor counseling, if a licensed Western blot has been performed as the additional, more specific test for reentry purposes, additional IFA testing may not be used to negate or resolve a positive or indeterminate Western blot result. For example, if a licensed Western blot test result is indeterminate, whether on the initial, substituted, or follow-up sample, a negative IFA result obtained subsequently is not sufficient to qualify a donor for reentry.

Products from prior donations by persons currently found to have repeatably reactive HIV screening tests may be released from quarantine based on a negative result of either a licensed IFA or Western blot. The results of additional

testing on the donor sample by either IFA or Western blot may be provided to the consignees of such products as a basis for the decision to trace and notify recipients of previously collected units from currently positive donors. In this context, use of the IFA to resolve Western blot indeterminate results is recommended.

2. Use of the Fluorognost HIV-1 IFA for screening

The IFA is not recommended for routine use as a screening test for blood and plasma donations. Errors may be more likely to occur in these settings because the test format is not conducive to handling large numbers of samples and because the test end-point is operator-dependent. Medical discretion should be used to decide whether the HIV-1 IFA test is indicated as a screening test in a particular instance because screening by the EIA is unavailable or impractical. Emergency situations involving donations of rare blood types, urgent screening of an HLA matched platelet donor, or urgent management of disasters might be examples when the IFA is appropriate.

When used for donor screening, the results of a single IFA test are sufficient to determine the HIV-1 status of the donor. Donors with reactive test results must be deferred, although they remain eligible for reentry. If the IFA is used as a screening test for HIV-1 antibodies, a different type of test, such as the Western blot, should be used as the additional, more specific test for validation of the presence of HIV-1 antibodies and for possible reentry. It should be noted by users that the licensed HIV-1 IFA test is not approved for detection of antibodies to HIV-2.

The format of the IFA lacks automated procedures and an objective read-out, therefore, results may be disposed to errors inherent in tests that depend on human judgement, both in reading and interpretation. For this reason, individuals reporting Fluorognost HIV-1 IFA results should have demonstrated proficiency at interpreting IFA results. The manufacturer provides both a Product Education Manual which contains a program of study, and a proficiency panel of coded samples for assessment of user proficiency. Each individual who intends to report IFA results is strongly advised by the manufacturer to qualify as a reader by completing this program of training and assessment.


for Kathryn C. Zoon, Ph.D.

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Date: April 23, 1992 Food and Drug Administration
Bethesda MD 20892

From: Director, Center for Biologics Evaluation and Research

Subject: Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products

To: All Registered Blood Establishments

INTRODUCTION

On September 25, 1991 the Food and Drug Administration (FDA) licensed the first enzyme immunoassay (EIA) for the simultaneous detection of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in human serum and plasma. This test, manufactured by Genetic Systems Corporation, is based on whole viral lysate antigens. Another combination EIA, based on recombinant antigens, and manufactured by Abbott Laboratories, received a license on February 14, 1992. These tests are indicated for detection of antibodies to HIV-1 and/or HIV-2 in human serum or plasma. The tests may be used for donor screening and as an aid in the diagnosis of potential infection with HIV-1 and/or HIV-2.

The availability of licensed screening tests for combined detection of antibodies to HIV-1 and HIV-2 provides blood centers with the capability of performing routine HIV-2 screening without the need to implement a second test in addition to the currently required test for antibodies to HIV-1. Based on this possibility, the issue of whether to recommend donor screening for HIV-2 was discussed at a meeting of the Blood Products Advisory Committee on September 27, 1991.

At the time of the meeting, only 31 cases of HIV-2 infection in the U.S.A. had been reported to the Centers for Disease Control, despite extensive surveillance studies. (One additional case has been reported since then.) These data indicated that HIV-2 infections presently represent only a minimal risk to the safety of the blood supply. Nevertheless, from a public health perspective, use of combination tests offers an opportunity to further protect the safety of blood recipients against HIV-2 infection without requiring an increased number of tests in blood centers. Donor screening for antibodies to HIV-2 would also reduce the need for continued surveillance studies which are expensive, difficult to sustain, and might nevertheless fail to prevent the earliest cases of HIV-2 transmission by blood products.

For these reasons, and with the concurrence of the Blood Products Advisory Committee, the FDA recommends that all establishments collecting whole blood, blood components, Source Plasma or Source Leukocytes implement a licensed test for detection of antibodies to HIV-2 by June 1, 1992. For this purpose the Agency finds acceptable either the use of a licensed combination test for detection of antibodies to HIV-1 and HIV-2 or the use of two separate tests licensed for detection of these antibodies.

To implement HIV-2 testing, modifications are necessary to the current recommendations for the prevention of HIV transmission by blood and blood products. The modifications affect donor selection and deferral procedures, the HIV reentry algorithm, Public Health Service recommendations for additional medical follow-up and counseling, and the management of potentially infectious units from prior collections. For this reason, the FDA is issuing a revised set of recommendations which replaces the Agency's memoranda to blood establishments dated February 5, 1990 and December 5, 1990.

The FDA further discussed donor deferral criteria for HIV at a meeting of the Blood Products Advisory Committee on March 13, 1992. Based on updated scientific data which were presented at this meeting, modifications have been made to some of the deferral criteria. The changes include a 12 month instead of lifetime deferral for sexual partners of persons with high risk behavior, and voluntary instead of recommended use of Confidential Unit Exclusion. Also, the FDA is discontinuing its recommendation for documentation in the donor record of the medical history of HIV associated signs and symptoms.

The revised memorandum also includes reference to use of a licensed immunofluorescence (IFA) test for antibodies to HIV-1 as an alternative to Western blot. On February 5, 1992, Waldheim Pharmazeutika, GmbH, Vienna, Austria was licensed to manufacture and distribute an HIV-1 IFA test which is labeled for use primarily as an additional, more specific test similar to previously licensed Western blot tests.

The revised memorandum does not address the practice of invalidation of aberrant and potentially incorrect screening test results. In past situations, FDA has taken the view that it is not appropriate to invalidate test results solely on the basis of an unexpectedly high rate of initial or repeat reactive tests. This is because of the possibility that a true positive sample with borderline reactivity could escape detection by a single instead of duplicate retest. Pending a specific recommendation, Blood Establishments are requested not to invalidate test results solely on the basis

of an unexpectedly high rate of initial or repeat reactivity in a test kit. The subject of test invalidation will be discussed at a meeting of the Blood Products Advisory Committee in the near future and specific recommendations in this area will be made following the public discussion.

In addition to the recommendations is an expanded section on "Exclusion/retrieval of potentially contaminated units from prior collections and notification of consignees." This section has been developed in accordance with recommendations of the Blood Products Advisory Committee which were obtained at a public meeting on January 17, 1991. Recommendations and regulations concerning recipient tracing and notification by Transfusion Services are under the authority of the Health Care Financing Administration (HCFA). It is expected that recipient tracing and notification should be carried out by Transfusion Services if the HIV-1 Western blot or IFA is positive on the current donor sample. If the HIV-1 Western blot or IFA is negative or indeterminate, but a second HIV-2 EIA (single virus or combination test) is repeatedly reactive, a medical judgement will be necessary regarding the potential benefits of recipient tracing, especially for units that were collected prior to June 1, 1992.

CLOSING REMARKS

Questions concerning these modified recommendations may be directed to the Food and Drug Administration, Center for Biologics Evaluation and Research, Division of Transfusion Science, Laboratory of Blood Bank Practices, via Telefax number 301-227-6431.

The recommendations contained in this memorandum may be implemented as soon as feasible, without prior approval from the Agency. Upon implementation, licensed blood establishments concurrently should submit a statement for their file indicating that revised standard operating procedures consistent with these recommendations have been put in place. The date for implementation of a routine screening test for HIV-2 should be documented at the blood center and in the license file.

To conform with these recommendations, manufacturers of licensed blood components for transfusion will need to submit an updated circular of information. Manufacturers of Source Plasma or Source Leukocytes will need to submit revised container labels.


for Kathryn C. Zoon, Ph.D.

**RECOMMENDATIONS FOR THE PREVENTION OF
HUMAN IMMUNODEFICIENCY VIRUS (HIV)
TRANSMISSION BY BLOOD AND BLOOD PRODUCTS**

Revised April, 1992

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**RECOMMENDATIONS FOR THE PREVENTION OF
HUMAN IMMUNODEFICIENCY VIRUS (HIV)
TRANSMISSION BY BLOOD AND BLOOD PRODUCTS**

Revised April, 1992

**I. AIDS EDUCATION, SELF-EXCLUSION AND CONFIDENTIAL UNIT
EXCLUSION**

A. Educational Information to Permit Self-Exclusion

All persons donating blood or plasma for transfusion or further manufacturing use should receive both written information and oral explanation about the safety of blood products in relation to AIDS epidemiology and the implications for donors who have engaged in certain high-risk activities. Donors should not be considered suitable unless information about these risks can be communicated in the language appropriate to each donor (including appropriate communication to persons with impaired vision or hearing) and the educational format is constructed to be culturally sensitive to promote comprehension. The procedures applied should provide an opportunity at each visit for the donor to consider the information and to make an informed and private decision about whether to donate. In some settings it is appropriate to use abbreviated materials for frequent, repeat donors such as autologous donors or serial Source Plasma donors who may be screened as often as twice in a seven day period, and who are familiar with the program employed in the establishment.

Appropriately trained blood establishment personnel should talk with each prospective donor about risk factors for HIV infection. The focus should be on behavior and not on stereotypes. For example, many men who have had male-to-male sexual experiences do not identify themselves as "homosexual," "gay," or "bisexual," but would identify with the description "sex with another man."

The direct questions concerning risk behaviors for HIV infection should be presented to potential donors orally, if possible. The technique demonstrated to be most effective by an FDA sponsored field trial included the use of an illustrated answer sheet for the donor to record a reply as the list of questions (see addendum) was read to the donor. The records for unsuitable donors do not need to include the answers to each of the direct questions about risk behavior, but may indicate that deferral was based on risk behavior history. If the questions are presented orally, the standard operating procedures should ensure that questions are not abridged and each donor record should indicate that satisfactory responses were received. Each donor record should also accurately reflect staff responsibility for this portion of donor screening procedures and document the decision concerning donor suitability or deferral.

The educational material on AIDS should also include a description of HIV associated clinical signs and symptoms including:

- Unexplained weight loss
- Night sweats
- Blue or purple spots typical of Kaposi's sarcoma on or under the skin, or on mucous membranes
- Swollen lymph nodes lasting more than one month
- Persistent white spots or unusual blemishes in the mouth
- Temperature greater than 100.5°F for more than 10 days
- Persistent cough and shortness of breath
- Persistent diarrhea.

It is not necessary for the donor record to include documentation of the donor history for HIV related signs and symptoms. It is necessary that donors be informed about HIV associated signs and symptoms so that they may self-defer if these conditions are present.

B. Criteria for the Exclusion of Unsuitable Donors Who Are at Increased Risk for HIV

The minimum information presented to potential blood and plasma donors at every visit should indicate clearly that persons meeting any of the following descriptions or having engaged in any of the following activities should not donate blood or blood components to be used for transfusion or further manufacturing:

- * Persons with clinical or laboratory evidence of HIV (AIDS virus) infection.¹
- * Men who have had sex with another man even one time since 1977.
- * Past or present intravenous drug users.
- * Persons with hemophilia or related clotting disorders who have received clotting factor concentrates.
- * Men and women who have engaged in sex for money or drugs since 1977

¹At this date, the most recent criteria from the CDC appear in MMWR 1987;36(S-1):1-9. A revision of these criteria is anticipated in the near future.

- * Persons who have had sex with any person meeting the above descriptions during the preceding 12 months.
- * Persons who have had, or have been treated for, syphilis or gonorrhea during the preceding 12 months, or who have had a reactive screening test for syphilis in the absence of a negative confirmatory test in the last 12 months. (Any synonyms for these diseases that may be appropriate to the local donor population should be included in the donor questionnaire and oral interaction.)

[Note: Refer to FDA's memorandum to all blood establishments dated December 12, 1991 for additional discussion.]

- * Persons who have received a transfusion of whole blood, a blood component (e.g. cryoprecipitated AHF, platelets) or a clotting factor concentrate (e.g., Factor IX, Human) within the past 12 months. Receipt of an FDA-licensed plasma derivative other than a clotting factor concentrate (e.g., Albumin [Human]) is not a basis for exclusion.
- * Persons born in or emigrating from countries where heterosexual activity is thought to play a major role in transmission of HIV-2 infection (i.e. sub-Saharan Africa and islands located near these areas of Africa²) and persons who have had sex with any person meeting the latter description.

[Note: These criteria should be discontinued upon implementation of a test for antibodies to HIV-2 no later than June 1, 1992.]

Sample direct questions on behavior related to increased risk of HIV infection are provided as an Addendum to this document.

During the health history interview, a donor may volunteer information about a specific instance of possible exposure to hepatitis viruses or to HIV. Suggested deferral periods for two such instances are provided below:

- * Persons who have been victims of rape during the preceding 12 months should be deferred.
- * Persons who have had contact with blood and body fluids through percutaneous inoculation (such as

²Sub-Saharan Africa includes all countries of Africa except Morocco, Mauritania, Algeria, Libya, Egypt, Tunisia, Sudan, Somalia and Western Sahara.

injury or accidental needlestick) or through contact with an open wound, non-intact skin, or mucous membrane during the preceding 12 months should be deferred.

Donors should also be informed that:

- * There is a time interval early in infection during which tests for HIV may be negative although an infection may still be transmitted.
- * A sample of blood will be tested for antibodies to HIV and the donor will be notified if a test is positive (see I.C.1.c); individuals with positive tests will be permanently deferred from future blood or plasma donation.
- * The names of people with repeatedly reactive tests for anti-HIV-1 or anti-HIV-2 will become part of donor deferral registries [as required by 21 CFR 606.160(e)].

Information concerning other mechanisms for obtaining HIV antibody tests should be readily available to individuals who may not qualify as donors but who present themselves as prospective donors because of concern about antibody status. Clear instructions concerning alternatives should be available to every prospective donor as well as information about tests to be performed and educational material identifying activities which threaten the safety of the blood supply.

It is also useful to have information readily available to every donor to facilitate prompt notification of the collection center in the event he or she becomes ill or decides the donation may have been inappropriate.

C. Second Exclusion Opportunity

Because of the extreme importance of the self-exclusion process to the safety of blood products, some blood establishments may find it useful to provide a second opportunity to prevent use of a unit from a high-risk donor.

1. Confidential Unit Exclusion (CUE)

Where peer pressure to donate voluntarily may compromise the self-exclusion process, an additional optional procedure can be used whereby a donor may indicate confidentially at the time of donation that his/her blood or plasma donation should not be transfused to others, or used for further manufacturing except as described in I.C.1.d. This procedure is most meaningful if it requires informed and knowledgeable action by every

donor, including autologous donors whose blood might be used homologously, and provides:

a) Strict confidentiality of the donor's decision and privacy in which to make the decision;

b) Assurances to the donor that confidentially excluded units will be tested and the donor notified of any positive test results;

c) Notification to the donor of the results, and counseling or appropriate referral of all donors with positive HIV antibody tests. The criteria for a positive test should be defined in the standard operating procedures of the establishment.

[Note: Pending the availability of licensed additional, more specific tests for anti-HIV-2, notification may be based on a repeatedly reactive HIV-2 EIA, as discussed below in Section II.]

d) Quarantine and destruction of all units designated not for clinical use except in a research protocol or in further manufacture of a special product specifically approved in writing by the Director, CBER. (See I.E.)

2. Private Interview

As another optional procedure to augment self-exclusion, a private interview may be conducted by a trained and competent health professional during which AIDS related educational information is presented orally and the opportunity for self-exclusion is offered. The interview approach has been preferable for paid donors who are unlikely to be responding to peer pressure and whose blood or plasma is intended for further manufacturing use rather than transfusion. Also, because confidential unit exclusion does not preclude donation, the interview technique may be preferred for donors about to undergo lengthy and expensive procedures such as plateletpheresis and granulocytapheresis. In these cases, early assessment of donor suitability may lead to deferral of the donor before the procedure.

D. Donor Consent

The donation records should include a signed consent statement with a provision equivalent in meaning to the following:

"I have reviewed and understand the information provided to me regarding the spread of the AIDS virus (HIV) by blood or plasma. If I am potentially at risk for spreading the virus known to cause AIDS, I agree not to donate blood or plasma for

transfusion to another person or for further manufacture. I understand that my blood will be tested for antibodies to HIV and other disease markers. If this testing indicates that I should no longer donate blood or plasma because of a risk of transmitting the AIDS virus, my name will be entered on a list of permanently deferred donors. I understand that I will be notified of a positive result."

An NIH Consensus Conference which was held in Bethesda, Maryland in July, 1986 determined that it is not ethical to fail to inform donors of test results which resulted in their deferral and/or will result in the discarding of their future collections. For this reason, the Agency believes that blood establishments should notify donors of all positive and indeterminate test results. If, despite the ethical considerations, a blood bank does not intend to notify donors of indeterminate results, the above statement should continue as follows:

"If, instead, the result of the testing is not clearly negative or positive, my blood will not be used and my name may be placed on a deferral list without my being informed, until the results are further clarified."

E. High-Risk Donors

Persons who have engaged in activities that put them at risk of HIV infection may not be donors. However, exemptions may be requested in the form of a specific license application or amendment to permit Source Plasma collection for special purposes, e.g., for research or for manufacture into in vitro products.

Applications requesting additional categories of exemption should include data documenting a need to use donors at increased risk of HIV for the intended product.

Collection of plasma from high risk donors as part of an Investigational New Drug (IND) application, requires a Source Plasma license application or amendment to be filed by the collection facility in addition to the IND filed by the manufacturer of the investigational product.

Special precautions are necessary for collecting, processing, labeling and shipping plasma from high-risk donors for any purpose. Advance approval from the Director, CBER is required for collection of such products [21 CFR 610.45(c)]. An outline of the precautions for handling plasma from high-risk donors may be obtained by writing to the Center for Biologics Evaluation and Research, Congressional, Consumer and International Affairs Branch (HFB-142), Room 1-58, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857. See also Section III.C. (Labeling and Use of Anti-HIV-Positive Products).

II. LABORATORY TESTING

A. Screening Tests

21 CFR 610.45, requires that blood and blood components intended for use in preparing a product for homologous transfusion or for further manufacturing use be tested according to the manufacturer's instructions and found negative by an FDA licensed test for antibodies to HIV (21 CFR 610.45). Conforming requirements were added to Parts 606, 610 and 640. Under authority defined in 21 CFR 606.140, as of June 1, 1992, the test(s) for antibodies to HIV are interpreted to include screening test(s) for antibodies to both HIV-1 and HIV-2.

Whole blood, blood components, or Source Plasma from donors whose samples are found to be repeatedly reactive for antibodies to HIV by an FDA-licensed HIV-1 EIA, an HIV-2 EIA, or an HIV-1/HIV-2 combination antibody screening test should be quarantined and either destroyed or diverted from use in transfusion or further manufacturing unless otherwise approved by the Director, CBER. Establishments which intend to ship units inadvertently collected from donors not known to be positive for anti-HIV must have FDA approved labels and comply with the reporting requirements of 21 CFR 610.45. Exception can be made for use of autologous blood according to recommendations published in memoranda to blood establishments dated March 15, 1989 and February 12, 1990.

The test for antibodies to HIV shall be performed pursuant to 21 CFR 610.45 (b). In all cases except emergencies [21 CFR 610.45 (a)], results must be available at the labeling location before release of the blood product. The blood establishment that collects the blood is responsible for assuring that appropriate records are maintained and that all FDA requirements are met.

The following terms apply to HIV antibody testing:

<u>Initially reactive</u>	Initial EIA test is reactive.
<u>Repeatedly reactive</u>	One or both duplicate EIA retests is (are) reactive.
<u>Negative</u>	Initial EIA test is negative or if reactive, both duplicate EIA retests are negative.
<u>Positive</u>	Repeatedly reactive EIA test; Western blot or IFA positive.
<u>Indeterminate</u>	EIA repeatedly reactive; Western blot or IFA neither positive nor negative.

B. Medical Follow-Up, Counseling and Donor Deferral Policies

1. Medical Follow-Up and Counseling

- a. The Public Health Service (PHS) has made the following recommendations¹ in regard to testing for anti-HIV-1:**

All samples repeatedly reactive for anti-HIV-1 by EIA should be further tested to determine whether the donor is truly positive. These additional tests (e.g., Western blot or IFA) may be done before informing donors of their repeatedly reactive test results.

Because in a blood donor population approximately 90% of repeatedly reactive anti-HIV-1 EIA results are falsely positive, some facilities have delayed notification of persons when additional, more specific tests are not clearly positive, i.e., are indeterminate. Notification of individuals repeatedly reactive for anti-HIV-1 but with indeterminate Western blot or IFA results is recommended because these donors are included in donor deferral registries. This notification should contain information about the significance of the test results, and suggest medical follow-up. If indeterminate patterns are stable for six or more months, in the absence of risk factors, clinical symptoms, or other findings, the individual may be considered negative for purposes of counseling. However, these individuals should be advised that they are not acceptable as donors because of their indeterminate test results. (Donor reentry criteria may apply in some cases, as described in II.C.)

Follow-up of positive results is important both to the public health and to the individual who may be expected to modify behavior to protect intimate contacts. It is strongly recommended, therefore, that donors be notified of all positive test results, and that reporting of results occur in the context of medical counseling.

- b. The following points summarize additional PHS recommendations which concern follow-up testing, notification and medical counseling of individuals with repeatedly reactive combination screening tests for anti-HIV-1/HIV-2. (see Figure 1):**

Supplemental testing for both HIV-1 and HIV-2 antibodies should be performed prior to notification and counseling. For

¹The PHS recommendations are repeated here for purposes of summarization only. (see MMWR 1985;34:1-5, MMWR 1987;36:509-15, MMWR 1988;36:833-840 & 845, MMWR 1989;38:5-7.)

persons found to be HIV positive for the first time, medical counselors may recommend that a fresh specimen be obtained to verify reproducibility of the test result.

Supplemental testing for HIV-1 should include an additional, more specific test (currently a Western blot or IFA).

- i. If a positive result is obtained, the presence of antibodies to HIV is presumed and the donor may be notified and counseled, as described in B.1.a., regardless of the actual virus type.

For surveillance purposes, additional, more specific testing for HIV-2 can be performed under research conditions on donors who are confirmed positive for HIV-1 antibodies by a licensed Western blot or IFA and who have epidemiological risk factors for HIV-2. These apply to persons born in or emigrating from Sub-Saharan Africa and the nearby islands and to sexual partners of these persons or of known HIV-2 infected persons, as well as to offspring of women in these groups.

- ii. If the HIV-1 Western blot or IFA is negative or indeterminate, a licensed EIA test specific for antibodies to HIV-2 and different from the test used for screening should be performed.

A negative HIV-2 EIA test result may be interpreted to exclude infection with HIV-2. The donor may be notified and counseled based on the HIV-1 test results (see B.1.a.)

A repeatedly reactive HIV-2 EIA test result may indicate infection with HIV-2. Medical judgement is required to decide what approach to take toward counseling and additional testing:

The index of suspicion for HIV-2 infection may be based on the presence of epidemiological risk factors for HIV-2, as noted above in B.2.a., or on the specific indeterminate pattern of gag plus pol bands on the HIV-1 Western blot.

Research studies suggest that investigational tests for HIV-2 antibodies such as Western blot, RIPA, IFA, and synthetic peptide-based EIA may be of value in the interpretation of the HIV-2 screening test result and in providing information useful for counseling the donor. Such supplemental tests should be used routinely when they become available commercially under FDA license.

- iii. If a screening test for antibodies to HIV-2 is performed as part of the initial HIV test and is found to be repeatedly reactive, the performance of a second HIV-2

EIA of a different type (either single virus based or HIV-1/HIV-2 combination test) may be useful prior to notification and counseling. If a negative result is obtained by the second HIV-2 EIA, the likelihood of HIV-2 infection is remote. If the second test is also repeatedly reactive, then medical judgement is required to decide what approach to take toward counseling and additional testing, as discussed in the preceding section.

2. Donor Deferral

Donors whose blood samples are found to be repeatedly reactive by screening tests for antibodies to HIV-1, HIV-2 or both viruses should be indefinitely deferred. In some cases, donors may meet suitability criteria for reentry (see below.) Repeatedly reactive screening test results and positive results from additional, more specific tests should be permanently recorded in a way that the donor can be identified subsequently as permanently deferred without disclosing the reason to unauthorized personnel.

Negative anti-HIV test results may be recorded permanently in the individual donor record or the information may be maintained separately, but should be readily available within the collecting facility for verification of negative tests on prior donations. Confidentiality of the results should be protected while, at the same time, it should be ensured that products from unsuitable donors are excluded from use. The deferral system should also ensure that products obtained from subsequent donations of unsuitable donors will not be distributed [21 CFR 606.160 (e)].

C. Donor Reentry

The following algorithm is recommended for reentry of blood donors previously deferred because of a repeatedly reactive test for antibodies to HIV-1 or HIV-2 when screening is performed by individual FDA-licensed HIV-1 and HIV-2 EIA tests, or a single combination test for anti-HIV-1 and anti-HIV-2. The recommended test sequence is outlined in Figure 2 and the criteria for reentry are summarized in Table 1.

A donor may be reentered when test results are as follows:

1. Recommendations for initial screening:

If a licensed HIV screening test is repeatedly reactive, an HIV-1 Western blot or IFA test should be performed on the initial sample. (It is preferable that licensed Western blot or IFA tests be used.) If the repeatedly reactive screening test was an HIV-2 test (single virus or combination test), and if the Western blot or IFA result is negative, then a second, licensed screening test for HIV-2 (either a single virus or combination test) which is different from the original HIV-2 test must also be performed.

- a. If a licensed HIV-1 Western blot or IFA is performed, it must be negative.
- b. If an unlicensed HIV-1 Western blot was performed, it must not have been positive by the criteria recommended by the Public Health Service (MMWR 1989; 38(S-7): 1-7). If, when indeterminate, the unlicensed Western blot had antibodies to any band among p24, p31, gp41, gp120 or gp160, then a licensed Western blot or IFA must be performed, and must be negative.
- c. The second (different) licensed HIV-2 EIA must be negative.
- d. If additional licensed screening tests are performed, they must be negative.

2. Recommendations for Tests on a Substituted Sample

If additional testing by Western blot, IFA or additional EIA testing for HIV-2 was not performed on the initial sample as required in 1., or if a licensed Western blot or IFA test proved necessary (1.b), but was not performed on the initial sample, reentry can still be considered provided that a newly obtained sample is "substituted" for the initial sample. Test results on the "substituted" sample must include:

- a. A negative licensed screening test using the same test kit (same manufacturer and same product) that was repeatedly reactive on the initial sample.
- b. A licensed Western blot or IFA result meeting the criteria described in 1.a or 1.b.
- c. A negative result of a second HIV-2 EIA (as described in 1.)
- d. If additional licensed screening tests are performed, they must be negative.

3. Recommendations for Follow-Up Testing

Follow-up testing must be performed on a newly obtained blood sample obtained at least six months later than the initial or "substituted" sample, whichever is later. Results of follow-up testing should be as follows:

- a. A screening test is negative using the same test kit (same manufacturer and product) with which the initial sample was repeatedly reactive. If the latter test kit is not a whole viral lysate based EIA for HIV-1 antibodies, then the sample must also be tested and found negative by a licensed whole viral lysate based HIV-1 EIA.

- b. A licensed HIV-1 Western blot or IFA is negative.
- c. A licensed screening test for HIV-2 (either a single virus or combination test) must be negative. If the screening test which was repeatedly reactive on the initial sample was a test for HIV-2 (single virus or combination test), then a second HIV-2 EIA, different from the original test, should be used on the follow-up sample.

4. Exception to the Algorithm

In cases where the test which was repeatedly reactive on the initial sample is no longer manufactured or distributed, it is permissible to test a substituted sample and a follow-up sample using another licensed screening test different from the one that was used on the initial sample.

III. LABELING

A. Products with Negative Anti-HIV Test Results

- 1. Products for transfusion: The instruction circular must state as required by 21 CFR 606.122(e) that the product was prepared from blood that was negative when tested for antibodies to HIV. Blood establishments should maintain a record of the date of implementation of testing for antibodies to HIV-2.
- 2. Products for Further Manufacturing Use: The container label should bear the statement, "Negative by a test for antibody to HIV" or equivalent statement. This statement is required by 21 CFR 640.70(a)(11) for Source Plasma and by 21 CFR 606.121(h)(3) for Recovered Plasma. If desired, the statement regarding HBsAg test results [21 CFR 640.70(a)(8)] and anti-HCV test results may be combined with the statement regarding the anti-HIV test results. An acceptable combined statement is, "Negative by tests for antibody to HIV and HCV and nonreactive for HBsAg."

B. Untested Blood and Blood Components

In emergency situations when there is no practical alternative to the release of blood or blood components for transfusion before completion of the required tests, the product(s) must be labeled and shipped in accordance with the provisions of 21 CFR 640.2(f) and 21 CFR 606.121(h).

In the case of rare products which cannot be tested because they were placed in frozen storage before anti-HIV tests were available and for which there is no available substitute product (e.g. rare red blood cell phenotypes), one of the following statements should be applied:

"CAUTION: This product was prepared before testing for antibodies to HIV was implemented and the anti-HIV status of the donor is not known," or

"This product was prepared before testing for antibodies to HIV was implemented. The donor was later tested on (Date) and found to be negative."

C. Units with Repeatedly Reactive Anti-HIV Test Results

There are currently few approved uses of anti-HIV reactive products other than for research, the preparation of HIV immune globulin/plasma, and the manufacture of reagents required for HIV testing. The collection of plasma for use in such products as well as their manufacture, special labeling and distribution requires advance approval of a specific license application or amendment by the Director, CBER. An outline of special procedures for labeling and distributing such products may be obtained from the Division of Transfusion Science, HFB-900, 8800 Rockville Pike, Bethesda, MD 20892.

Units which are not destroyed should be labeled with two cautionary statements as follows:

"Reactive by a test for HIV antibodies. The risk of transmission of HIV is present."

and

"For further manufacture into in-vitro diagnostic reagents for which there are no alternative sources" or "For laboratory research use only."

IV. EXCLUSION/RETRIEVAL OF POTENTIALLY CONTAMINATED UNITS FROM PRIOR COLLECTIONS AND NOTIFICATION OF CONSIGNEES

The FDA recommends excluding from use previously collected units of blood components or Source Plasma from any person who later exhibits signs or symptoms of AIDS, or later tests repeatedly reactive by a screening test for anti-HIV and does not have a negative licensed Western blot or IFA for antibodies to HIV-1 and a negative licensed EIA for antibodies to HIV-2.

A. Retrieval and Quarantine of Prior Collections

Blood centers should promptly (within 72 hours if possible) identify and quarantine in-date units from prior collections dating back five years whenever a donor has a repeatedly reactive screening test for antibodies to HIV, whether a test for anti-HIV-1, HIV-2, or a combination test. For plasma for fractionation, the identification and retrieval can be limited to units collected in the last six months which have not been pooled or further processed. The consignees of such units should be

notified so that the units that they hold can also be quarantined.

[Note: It is not intended that consignees should initiate recipient tracing based only on a repeatedly reactive donor screening test result and prior to the availability of the result of an additional, more specific test. The purpose of this first notification is to permit blood establishments to take control of units from prior collections so that they will not be transfused or used in further manufacturing pending the result of additional tests.]

B. Release of Units from Quarantine

Units from prior collections that are placed in quarantine may be released if the current (repeatedly reactive) blood, Source Plasma collection is tested by a licensed, more specific test for antibodies to HIV-1 (Western blot or IFA) and the result is negative. If the first, repeatedly reactive screen was a combination test for antibodies to HIV-1/HIV-2, or an individual test for anti-HIV-2, then a second, different licensed HIV-2 EIA (either single virus or combination test) must also be negative.

If the collection occurred more than one year prior to the donor's most recent negative screening test(s) for antibodies to HIV-1 and HIV-2, release can also occur. If the most recent negative screening test for HIV was obtained prior to June 1, 1992, then a negative screening test for HIV-1 alone is sufficient to establish the relevant time period.

C. Notification of Consignees of Additional Test Results

Blood centers should notify consignees of units obtained from the donor's prior collections of the results of additional testing on the donor's current sample. Such testing includes the results of an HIV-1 Western blot or IFA and, if the performed screening included a test for HIV-2, the result of a second EIA for HIV-2. This testing and notification should be completed as soon as feasible (within two weeks if possible) following the repeatedly reactive screening test.

Notification of consignees is performed so that Transfusion Services can carry out recipient tracing and notification (through physicians) if the HIV-1 Western blot or IFA is positive. If the HIV-1 Western blot or IFA is negative or indeterminate, but a second HIV-2 EIA is repeatedly reactive, a medical judgement should be made regarding the potential benefits of recipient tracing. When commercially available, additional tests (other than EIA) for antibodies to HIV-2 may be useful in making this decision.

Figure 1

**PUBLIC HEALTH SERVICE RECOMMENDATIONS
FOR ADDITIONAL TESTING AND NOTIFICATION BASED ON
COMBINATION SCREENING FOR HIV-1 AND HIV-2**

**REPEATEDLY REACTIVE COMBINATION TEST
[DISCARD UNIT, PLACE DONOR NAME ON DEFERRAL LIST]**



PERFORM LICENSED HIV-1 WESTERN BLOT OR IFA

**POSITIVE
[PERMANENTLY DEFER DONOR]**

NEGATIVE OR INDETERMINATE



NOTIFY AS POSITIVE FOR HIV

**PERFORM HIV-2 EIA
(SINGLE VIRUS BASED TEST)**



NEGATIVE

REPEATEDLY REACTIVE

**TEST FOR HIV-2
BASED ON RISK FACTORS**



**NOTIFY BASED ON
HIV-1 TEST RESULTS**



**NOTIFY OF RESULTS
CONSIDER RESEARCH
TESTS FOR HIV-2**

Figure 2

FDA RECOMMENDATIONS FOR DONOR REENTRY FOLLOWING A REPEATEDLY REACTIVE SCREENING TEST FOR HIV

REPEATEDLY REACTIVE EIA

LICENSED HIV-1 WESTERN BLOT OR IFA

POSITIVE
OR INDETERMINATE

NEGATIVE

NOT DONE

NO REENTRY

SECOND, DIFFERENT HIV-2 EIA
REQUIRED IF ORIGINAL
REPEATEDLY REACTIVE EIA
WAS AN HIV-2 TEST

TEST SUBSTITUTED SAMPLE
BY ORIGINAL EIA

REPEATEDLY
REACTIVE

NEGATIVE

REPEATEDLY REACTIVE

NEGATIVE

NOT DONE

NO REENTRY

NO REENTRY

TEST FOLLOW-UP SAMPLE
AT ≥ 6 MONTHS BY:

- ORIGINAL EIA
- WHOLE VIRAL HIV-1 EIA
- LICENSED HIV-1 WESTERN BLOT OR IFA
- HIV-2 EIA (SECOND, DIFFERENT TEST REQUIRED
IF ORIGINAL EIA WAS AN HIV-2 TEST)

ALL TESTS NEGATIVE

ANY TEST REPEATEDLY REACTIVE,
POSITIVE OR INDETERMINATE

REENTER DONOR

NO REENTRY

*Special considerations apply to the use of an unlicensed Western blot on the original or substituted sample. See section II.C.1.b. in the text.

Table 1

HIV ANTIBODY TEST RESULTS QUALIFYING A DONOR FOR REENTRY

Test Results

Time	Screening Tests				Western Blot (WB) or IFA	Second HIV-2 EIA
	A	B	C	D		
Initial	RR	ND or NEG	ND ^a or NEG	ND or NEG	ND ^b , NEG or INDET (Licensed WB or IFA required if unlicensed WB has a band to p24, p31, gp41, gp120 or gp160. Licensed WB or IFA must be NEG.)	ND ^a or NEG
Sub- stituted	NEG	ND or NEG	ND ^a or NEG	ND or NEG	same as initial sample	NEG
Follow-Up ≥ 6 mos.	NEG	ND ^d or NEG	ND ^a or NEG	ND or NEG	NEG (must be a licensed WB test)	NEG

- ^a If test A is not an HIV-2 EIA or combination test, then an HIV-2 EIA is required
- ^b Substituted sample required if test is not done.
- ^c Must perform if test A is an HIV-2 EIA (either a single virus or combination test.) If a second HIV-2 EIA is necessary, but was not done, then such a test should be performed on a substituted sample.
- ^d Must perform if test A is not a whole viral lysate based EIA test for HIV-1.

See next page for terms and abbreviations used in this table.

Terms and abbreviations used in the table are:

Screening test A	The licensed screening test for HIV-1, HIV-2 or both which is repeatedly reactive on the original sample.
Screening test B	A licensed screening test for HIV-1 different from test A and based on a whole viral lysate antigen.
Screening test C	A licensed screening test for HIV-2 other than test A or test B.
Screening test D	A licensed screening test for HIV other than test A, B or C.
Second HIV-2 EIA	A licensed screening test for HIV-2 that is different from test A (may be a single virus or combination test)
Western blot, WB	A licensed or unlicensed Western blot test for anti-HIV-1.
IFA	A licensed immunofluorescence assay for anti-HIV-1.
ND	Test not done. Performance of the test is optional unless otherwise noted.
RR	Repeatedly reactive screening test.
NEG	Negative test result.
INDET	Western blot test result is indeterminate. CDC criteria apply to unlicensed tests.

ADDENDUM

Sample Direct Questions on High Risk Behavior:

1. Do you have AIDS, or have you ever had a positive test for the AIDS virus (HIV)?
2. Have you ever taken illegal drugs with a needle, even one time?
3. Have you ever taken clotting factor concentrates for a bleeding disorder such as hemophilia?
4. Were you born in or did you move to this country from the part of Africa south of the Sahara desert or the islands close to that part of Africa?
5. Have you had sex with anyone who was born in or moved to this country from the part of Africa south of the Sahara desert or the islands close to that part of Africa?
6. At any time since 1977, have you taken money or drugs for sex?
7. Male donors: Have you had sex with another man, even one time since 1977?
8. Have you had sex in the last 12 months with anyone who has had AIDS or has had a positive test for the AIDS virus?
9. Female donors: In the last 12 months, have you had sex with a man who had sex, even one time since 1977, with another man?
10. Have you had sex in the last 12 months with anyone who has ever taken illegal drugs with a needle?
11. At any time in the last 12 months, have you given money or drugs to anyone to have sex with you?
12. At any time in the last 12 months, have you had sex with anyone who has taken money or drugs for sex ?
13. Have you had sex in the last 12 months with anyone who has taken clotting factor concentrates for a bleeding disorder such as hemophilia?
14. In the last 12 months, have you had syphilis or gonorrhea, or have you been treated for syphilis or gonorrhea? (Add locally appropriate synonyms.)

15. In the last 12 months, have you received blood or blood products by transfusion for any reason, such as an accident or surgery?

• Questions 4 and 5 should be omitted following implementation of an FDA-licensed test for antibodies to HIV-2.

[DOCKET NO. 91N-0467]

DRAFT

POINTS TO CONSIDER IN THE
DESIGN AND IMPLEMENTATION OF FIELD TRIALS FOR
BLOOD GROUPING REAGENTS AND ANTI-HUMAN GLOBULIN

1st draft 1992

For further information about this draft, contact:

Center for Biologics Evaluation and Research (HFB-940)
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD 20892
301-227-6487.

Submit written comments on this draft to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
Rm. 1-23
12420 Parklawn Drive
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Submit requests* for single copies of this draft to:

Congressional, Consumer, and International Affairs Branch (HFB-142)
Food and Drug Administration
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* except that written requests delivered by carriers other than the U.S.
Postal Service for single copies of this draft should be submitted to:

Congressional, Consumer, and International Affairs Staff (HFB-142)
Food and Drug Administration
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7564 Standish Place
Rockville, MD 20855.

Comments and requests should be identified with the docket number found in
brackets in the heading of this document.



Date: April 23, 1992

From: Director, Center for Biologics
Evaluation and Research

Subject: Exemptions to Permit Persons with a History of Viral
Hepatitis Before the Age of Eleven Years to Serve as
Donors of Whole Blood and Plasma: Alternative
Procedures, 21 CFR 640.120

To: All Registered Blood Establishments

This Memorandum responds to inquiries that the FDA has received for exceptions from certain donor suitability requirements set forth in 21 CFR 640.3(c)(1) and 640.63(c)(11). These two regulations preclude persons with a history of viral hepatitis, occurring at any age, from donating Whole Blood or Source Plasma.

On September 27, 1991, after discussion in a public meeting, the Blood Products Advisory Committee to the FDA recommended that the regulations referenced above be amended because they exclude a large number of suitable donors. The Committee further recommended that persons with a history of viral hepatitis, prior to 11 years of age, not be excluded from donating Whole Blood and Source Plasma.

In accordance with the recommendation of the Committee, FDA intends to propose amendments to the regulations referred to above. Until the regulations are amended, the FDA will consider procedures acceptable that permit a person with a history of viral hepatitis before the age of eleven years to serve as a donor of Whole Blood and Source Plasma. This exception to 21 CFR 640.3(c)(1) and 640.63(c)(11) is in conformance with 21 CFR 640.120, Alternative Procedures.

Kathryn C. Zoon
for Kathryn C. Zoon, Ph.D.

DRAFT

POINTS TO CONSIDER IN THE DESIGN AND IMPLEMENTATION OF FIELD TRIALS FOR BLOOD GROUPING REAGENTS AND ANTI-HUMAN GLOBULIN 1ST DRAFT 1992

In response to concerns expressed over the intent in performing field trials on new Blood Grouping Reagents and Anti-Human Globulin Reagents and the subsequent impact on field trial design and implementation, a portion of the November 1990 workshop, "Reagents for the 1990's" was devoted to the discussion of these parameters. A consensus was reached on many elements and the major points of concern have been incorporated into this document. It should be noted that these products have not required the filing of an Investigational New Drug Application (IND) for conducting field trials.

PURPOSE OF FIELD TRIALS - Field trial testing of blood grouping reagents and anti-human globulin reagents is performed after the manufacturer has documented specificity and potency. It is useful primarily to show that the directions for use are adequate, that a broad cross-section of unselected samples give expected results, and that the product performs as expected in the hands of routine users rather than experts.

DESIGN OF FIELD TRIALS - Trials should be constructed to adequately represent the situations and sample mix in which the product will be used.

DRAFT

I. GENERAL

- A. Field trials should commence only after the manufacturing methods and performance criteria are well established and the manufacturer has determined the recommended methods for use and established that the reagent achieves accurate and reproducible results, i.e.:
1. The reagent has been assessed in-house or through contract by a minimum of 300 tests.
 2. Potency is equal to or greater than the reference preparation.
 3. The reagent is specific by all package insert methods. A panel of appropriate rare cells should have been employed.

II. SAMPLES/SITE SELECTION

A. Reagents

1. Reagent under test
 - a. at least 2 lots
 - b. made by the method described in the application for license
 - c. at least 1 lot must be a batch intended for distribution
2. Approved referee reagent (currently marketed, licensed product or other product approved by FDA¹)
 - a. must meet or exceed an approved polyclonal (unless a new, unique product)
 - b. an approved monoclonal is optional, in most cases
3. Blind coded

¹ If a licensed product is not available for use as a referee reagent, approval from FDA to use an unlicensed product for this purpose may be requested.

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B. Representative Sites

1. Usually at least 3 (excluding the manufacturer's own laboratories)
2. Cover different geographic regions
3. Cover population distributions
4. Cover different facility sizes and procedures
5. Cover different facility functions
 - a. blood collection establishment
 - b. transfusion service
 - c. clinical laboratory
6. Sites outside of the US are acceptable if US standards (i.e., CGMP's) are followed in protocols and recordkeeping.

C. Number of tests

Generally, sample size should be chosen to ensure that, statistically, a minimum of 10% of the samples will be either negative for the antigen corresponding to the antibody under test or positive for the antigen corresponding to the antibody under test with exceptions for very rare specificities.

1. Unselected samples for qualifying new ABO or Rh₀(D) reagents - normal donor samples
 - a. manual or microplate - 3,000 - 5,000 if no problems are encountered
 - b. automated - routine production samples tested for 5 days by at least two operators
2. Selected samples - unusual samples
 - a. additional manual tests - each of the selected samples listed in D. below

² Smaller numbers may be appropriate for reagents other than ABO and Rh₀(D).

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- D. Selected samples - must represent the patient/donor population

100 of each of the following

1. Variations in collection and storage
 - a. Clotted samples
 - b. Anticoagulated samples (cover each that is recommended)
 - c. Fresh samples
 - d. Frozen/deglycerolized cells¹
 - e. Stored samples (at 28 days and/or the anticoagulant expiration date, whichever is longer)
 - f. enzyme treated cells, if not prohibited by labeling
2. Variations in donor/patient age
 - a. elderly people (> age 80)
 - b. cord samples
3. Ethnic groups/known variants
 - a. Variable, depending on reagent under test
 - ex. Oriental (B_{sub}) for ABO
 - Black (D variants) for D
 - b. Unselected Caucasian, Black, Hispanic, Asian

¹ Fewer than 100 samples may be used if a smaller number can be shown to be adequate.

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10 - 25 of each of the following

1. Disease groups
 - a. acquired antigens, if pertinent
 - b. serum protein abnormalities
 - i. multiple myeloma
 - ii. Waldenstrom's macroglobulinemia
 - iii. pregnant women
 - c. autoimmune hemolytic anemia
 - d. oncology patients
 - i. lymphomas
 - ii. leukemias
2. Interfering substances
 - a. polyagglutinable cells
 - b. positive direct antiglobulin test
 - c. lipemic sample
 - d. hemolyzed sample
3. Other
 - a. Exalted expression of the antigen
 - b. Weakened expression of the antigen

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- E. Methods - all methods/specimens listed in package insert
 - 1. All sites by tube test
 - 2. At least 1 site by slide⁴
 - 3. At least 1 site by microplate⁴
 - 4. At least 1 site by automated method⁴
- F. Records
 - 1. Clearly defined protocol
 - a. define consistent recording procedures
 - b. include detailed analysis of discrepancies
 - c. maintain documentation of known samples and sample sources
 - 2. Complete
 - 3. Consistent with GMP's
- G. Follow-up
 - 1. Discrepancies must be reported to the manufacturer immediately and studied by the manufacturer or a referee laboratory.
 - 2. Aberrant samples should be stored for reference and submitted to FDA if requested.

⁴ If applicable.

[DOCKET NO. 91N-0466]

DRAFT

POINTS TO CONSIDER IN THE
MANUFACTURE OF IN VITRO MONOCLONAL ANTIBODY PRODUCTS
FOR FURTHER MANUFACTURING INTO
BLOOD GROUPING REAGENT AND ANTI-HUMAN GLOBULIN

March 1992

For further information about this draft, contact:

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301-227-6487.

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* except that written requests delivered by carriers other than the U.S. Postal Service for single copies of this draft should be submitted to:

Congressional, Consumer, and International Affairs Staff (HFB-142)
Food and Drug Administration
Suite 109, Metro Park North 3
7564 Standish Place
Rockville, MD 20855.

Comments and requests should be identified with the docket number found in brackets in the heading of this document.

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POINTS TO CONSIDER IN THE MANUFACTURE OF *IN VITRO* MONOCLONAL ANTIBODY PRODUCTS FOR FURTHER MANUFACTURING INTO BLOOD GROUPING REAGENT AND ANTI-HUMAN GLOBULIN

MARCH 1992

In follow-up to the 7-9 November 1990 workshop "REAGENTS FOR THE 1990's", the comments received from licensed manufacturers of Blood Grouping Reagents, users, and other interested parties concerning the pertinent "Points to Consider Documents" were reviewed and incorporated into this document.

Subsequent to the publication of the Federal Register Notice of Availability, all licensed manufacturers and other interested parties will have the opportunity to review and comment on the draft.

Specific references to the pages which your comments relate to or photocopies of the pages with your comments written on them would be helpful and appreciated. Responses are requested within 60 days of the date of publication of the Federal Register Notice of Availability, however, comments are welcome at any time.

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POINTS TO CONSIDER IN THE MANUFACTURE OF *IN VITRO* MONOCLONAL ANTIBODY PRODUCTS FOR FURTHER MANUFACTURING INTO BLOOD GROUPING REAGENT AND ANTI-HUMAN GLOBULIN

MARCH 1992

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POINTS TO CONSIDER IN THE MANUFACTURE OF *IN VITRO* MONOCLONAL ANTIBODY PRODUCTS FOR FURTHER MANUFACTURING INTO BLOOD GROUPING REAGENT AND ANTI-HUMAN GLOBULIN¹ MARCH 1992

Introduction

This draft reflects comments from the 7-9 November FDA workshop, "Reagents for the 1990's". Since 1983, the former "Points To Consider" (PTC) documents regarding monoclonal antibodies have been used by blood grouping reagent manufacturers primarily as guidance in seeking FDA licensure of new blood grouping reagents of monoclonal origin. Other "Points to Consider" documents are now available as well (See Appendix I).

Blood grouping reagents¹ are perhaps unique in that the serological tests available for evaluating the finished products are known to be particularly effective in predicting their usefulness. The intermediate products intended for further manufacturing use, however, can be assessed only in relation to good manufacturing practices and for conformance with criteria that have been shown to yield a satisfactory finished product. Therefore, this document is intended to apply primarily to the products for further manufacturing use, which are licensed by the Center for Biologics Evaluation and Research for use in concert with a final manufacturer of blood grouping reagents. In order for the manufacturer of a monoclonal antibody to obtain a U.S. license for the product for further manufacturing use, that manufacturer must enter into an agreement with a final product manufacturer to obtain a U.S. license for the final product. This agreement is known as a shared manufacturing agreement. Policy on this type of arrangement is currently being developed and is not yet finalized. The extent to which all of the particulars in this document will apply in an establishment

¹ Throughout this document, "blood grouping reagents" is being used as a generic term for those products with the proper names "Blood Grouping Reagent" and "Anti-Human Globulin".

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engaged in complete manufacturing of a final product may vary in some circumstances. However, if elements of this document are not satisfied, the manufacturer should demonstrate that equivalent procedures have achieved the same objectives or that there is no need for such procedures.

In addition, earlier FDA guidance (such as the June 1983 "Points to Consider in the Manufacture of *In Vitro* Monoclonal Antibody Products Subject to licensure") concerning the information necessary for monoclonal antibody product approval has not always made a clear distinction between those items that are essential for demonstrating qualification of each lot and other items essential only to the license application or amendment process for new products. To reduce confusion concerning this point, a new section has been added at the end of this draft document to list in a broadly generic context those items which should be documented in the records for each bulk lot of monoclonal antibody produced for further manufacturing use. Where additional explanation is required, the reader should refer to other guidance such as the "recommended methods," or other references for more information.

This list is not intended to detail all of the elements of a manufacturing record that would meet good manufacturing practices requirements. The particular process used by each manufacturer should be reviewed for the purpose of identifying other critical information pertinent to each specific product.

Final product requirements and test methods for blood grouping reagents are described in 21 CFR Part 660 and the applicable "recommended methods" documents. The pending revisions of "recommended methods" documents also include new sections concerning obtaining exceptions from statutory and regulatory requirements and additions appropriate for products made from monoclonal antibody source material. Comments are welcome concerning additional modifications that may be applicable to the monoclonal products.

The amount of data necessary to support labeling claims and demonstrate specific activity of a new reagent is not completely described at this point. A major section of the 7-9 November workshop was devoted to achieving consensus on this important point and a draft PTC for field trials has been prepared for comment.

It should be noted that the FDA regulatory scheme provides for significant flexibility in regard to these products. The guidance included in documents such as this one describes the

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usual case based on past experience. Although it is always necessary to document that a new product is safe and effective, there are many alternative ways of achieving this objective.

For example, a typical new product would be developed and at least 3 lots characterized by the manufacturer as to avidity (if applicable), potency, specificity, and stability. Each of the 3 lots should be produced from a separate batch of antibody beginning at the stage of thawing frozen aliquots of a manufacturer's working cell bank.¹ It is not necessary to show separate cloning procedures, etc. Field trials would then normally be conducted with 2 or more lots (1 of which is a full scale production size lot) in 3 representative sites utilizing the new product in parallel tests with an FDA licensed product or other reference material approved by FDA².

The field trials traditionally serve primarily to demonstrate that the new product performs adequately in a variety of circumstances, that directions for use can be applied by the average laboratory worker, and that tests with broad, unselected donor and patient groups give accurate results comparable to those obtained in the manufacturer's own laboratory. However, the extent to which specificity must be assured by the manufacturer's own laboratory testing will be partially dependent upon the availability of information concerning past experience with the same antibody (i.e., from the same clone, produced by identical methods) and the design and size of field trials. If the protocol for the field trials is adequate to ensure that good manufacturing practices for testing and recordkeeping are applied, and all aberrant findings are refereed, the field trial data may also support specificity claims. The number of random samples to be tested may also be reduced by appropriate selection of known cell panels and historical information about other use of the same antibody for equivalent purposes.

¹ The Master Cell Bank (MCB) is defined as a quantity of cells derived from a single tissue and stored at -70°C or below in aliquots which would be used for the production of the Manufacturer's Working Cell Bank (MWCBC).

² If a licensed product is not available for use as a reference material, approval from FDA to use an unlicensed product for this purpose may be requested.

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General Concepts

The following information concerning *in vitro* diagnostic products produced from monoclonal antibodies provides additional guidance on applicable requirements of 21 CFR Parts 600-660 and Parts 800-820. The documents "Recommended Methods for Blood Grouping Reagents Evaluation" and "Recommended Methods for Anti-Human Globulin Evaluation" are also available from CBER and should be consulted for information on testing finished products, especially for more information on defining specificity. Potency requirements for some polyclonal products have been very minimal due to scarcity of source materials (e.g., anti-Jk^b, anti-Fy^b, etc.); however, since this is not an issue with monoclonal products, additional or higher requirements may apply. Similarly, for a monoclonal antibody product or one produced from a blend of monoclonal antibodies, the licensing or other premarket process may require additional information in regard to specificity because the chance of encountering an unusual or more limited pattern of specificity is significantly greater. Likewise, stability characteristics vary more with each clone and the particular growth conditions, so more stability documentation is appropriate as compared to traditional products prepared from large pools of human polyclonal source material which rarely deviated from the expected norms. With additional experience it should be possible to better define the important parameters and to further minimize the performance of tests which do not predict quality. We encourage the submission of alternative ideas for reducing effort while maintaining product quality.

I. CHARACTERIZATION OF CELL LINES

Although serological tests on final products deserve the primary emphasis for *in vitro* diagnostic products such as blood grouping reagents and anti-human globulins, background information on production cell lines and the derivation of clones should be available. The summary of the characterization of a cell line used to produce *in vitro* diagnostic products will usually include a brief description and applicable data concerning:

1. the origin and culture history, including cloning and recloning procedures;

* These and all other documents referenced in this document are available from Congressional, Consumer, and International Affairs Staff (HFB-140), Food and Drug Administration, 5600 Fishers Lane, Metro Park North, Room 109, Rockville, MD. 20857.

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2. methods and materials pertinent to maintenance, propagation and storage of cell lines including the seed lot system and security measures;
3. growth characteristics and morphology;
4. markers relevant to purity and to cell line identification;
5. freedom from adventitious agents and their products (including in particular, mycoplasma).

NOTE: Comments concerning the potential usefulness of karyology for blood grouping reagents are welcomed.

II. PRODUCTION PROCEDURES

The production of antibody, whether it occurs in tissue culture or ascites fluids, should be under the control of the licensed manufacturer responsible for these steps, and all aspects should conform with applicable biologics and device requirements. The processes described in the license application should be the same as those used for production of the material referenced in the data and the samples submitted in support of the license application. Significant changes following licensure require license amendment and may require the submission of additional in-house and/or field trial data to show that safety, purity, potency, and efficacy have not been adversely affected. All relevant procedures should be outlined (complete standard operating procedures are not required to be submitted but should be available during inspections). A "flow chart" schematic may be useful to show the relationship of procedures to each other, to the facility layout, and to the organizational structure. Procedures outlined in the product license application (PLA) should include at least descriptions of the following:

1. the tissue culture procedure or inoculation, harvest, and passage procedures in mice of defined genealogy.
2. procedures for control of microbial contamination, including the measurement of process bioburden with alert and action limits.
3. the preparation of inocula, criteria for inocula, procedures for monitoring antibody production, and criteria and methods for harvest, including any applicable time limits.

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4. all purification, concentration or dilution steps performed on harvested antibody and acceptance criteria for each stage of manufacture.
5. all tests to be performed on product before marketing each lot and applicable specifications.

III. CHARACTERIZATION OF THE PRODUCT

1. Serological evaluation

The essential characterization of the blood grouping reagents and anti-human globulins will be primarily their performance in hemagglutination assays. (See last section on tests for each lot). If final products are to be prepared from blends of monoclonal antibodies, it will be necessary to characterize each of the components independently so as to fully understand the contribution of each component to the blend's performance. In addition, each clone should be described as part of an approved product license application or amendment. (Note: We are aware this is more restrictive than earlier discussions between FDA and industry. However, as discussed at the November workshop, based on additional scientific information, such procedures are important.)

The determination of the optimal conditions for final product reactivity should be fully described with respect to change observed over a range of pH values, protein concentrations, ionic strengths, incubation times, and temperatures. The product specifications developed, e.g., a pH limit of 6.7 to 7.3 should be based on data from such studies.

2. Immunologic, biochemical, or biophysical studies

It will be necessary to define the product in a way that permits assurance that spontaneous mutations affecting the immunoglobulin secretion have not occurred. To this end, it is usually useful to determine the immunoglobulin class and subclass and provide evidence that concurrent production of minor populations of additional light or heavy chains is not occurring.

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We are not aware of any biochemical or biophysical criteria which are more sensitive than hemagglutination in assuring product specificity and reactivity for blood grouping reagents. However, to the extent that profiles may be useful in establishing homogeneity and in evaluating a product over time, characterization by some of the following techniques (if appropriate references are used) may provide a useful adjunct to serological findings.

- a) The electrophoretic migration of the isolated and purified antibody product in both the native and reduced states measured relative to the standard material on polyacrylamide gels. Coomassie blue staining has been shown to be useful in quantitation studies while silver staining has been shown to be more sensitive in detecting impurities. For certain products densitometric scans of the gels is required. Contaminant limits should be established to ensure lot to lot consistency.
- b) The spectrotpe of purified antibody from each lot could be compared with a reference material by isoelectric focusing.
- c) Protein concentration of purified solution as established by a suitable assay (for example, a Lowry determination), may be useful in demonstrating that antibody excretion is consistent from one batch to the next.

IV. STABILITY OF THE PRODUCT

The final product will be subjected to a variety of conditions when used in the field. Therefore, tests should be done to establish a dating period during which consistent results can be expected under realistic field conditions. If six months of satisfactory stability data on the finished product are available at the time of licensing, the remaining stability studies usually may be performed concurrent with distribution and a longer dating period (up to 4 times the "real-time" data) may be considered.

A typical pre-licensing stability study, for either intermediate or final product, would include tests at three month intervals throughout the maximum shelf life of the product and at least 1 year beyond. Serial titrations should be used when necessary to permit meaningful comparison of reactivity.

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In addition to pre-licensing stability studies, on-going stability studies should be fully described in the PLA.

Retention samples should be available throughout the dating period for each lot manufactured. At least two, 10 ml aliquots in sterile containers are recommended. The container material, type of closure, and storage conditions should be the same as for marketed product. Labels may be attached by tie tag and should accurately reflect the labeling of marketed products.

See the next section for additional information on stability test procedures. Potency evaluation is the only routinely required parameter for stability studies.

The date of manufacture (DOM) requires definition to ensure consistent meaning for dating periods. The DOM will be defined as the date first harvesting of antibody begins (21 CFR 610.50(b)(2)). The date of the last valid potency test (21 CFR 610.50(a)) has no meaning for these products. All limits on time and temperature for product storage after the DOM require definition for each step in manufacture.

V. TESTS APPLICABLE TO EACH LOT OF MONOCLONAL ANTIBODY FOR FURTHER MANUFACTURING USE

1. Antibody content (potency)

The amount of antibody present should be determined to be within acceptable limits. Protein concentration is not a suitable measurement for this purpose. All tests should include comparison to a known stable control or other appropriate in-house reference preparation. If hemagglutination assays are used, reproducibility of the method should be documented before beginning the stability protocol. Variability of results should be minimized by appropriate application of accepted practices such as:

- a) When assaying high titer products use intermediate direct dilutions to reduce the number of doubling serial transfers to less than 10.
- b) Choose tube sizes and assay volumes that permit thorough mixing with ease.
- c) Use the same antigen source, precise cell concentrations, and narrow limits for age of antigen samples and/or compensate for individual

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antigen variability by choosing phenotypes least vulnerable to variation and averaging results and/or use pools of antigen from 3 or more individuals.

- d) Perform titrations in duplicate or triplicate and average results and/or average results from different operators.
- e) Define clear endpoints and use a visual comparison chart when evaluating results.
- f) Consider using a scoring system that includes valuing reaction strengths observed as well as endpoints.
- g) Carry all titrations to a negative endpoint, i.e., a sufficient range of dilutions should be made to ensure that at least one, and preferably two, negative results can be observed beyond the endpoint chosen to define the titer value (e.g., 1+).
- h) Use an incubation period that can easily be reproduced. "Immediate spin" techniques are not acceptable.
- i) Control all variables such as cell concentration, incubation temperatures, pH of diluents, resuspension techniques, etc.

NOTE: Variability of results greater than 2-fold differences in endpoint signals a reproducibility problem which should be resolved before stability protocols begin.

2. Antibody identity and specific reactivity

The test protocol should include antigens that establish the absence of serological contaminants and the unique identity of the product, i.e., distinguishes it from every other visually similar product prepared in that facility. See "recommended methods" for more details concerning initial product approval.

NOTE: Cord cells, black, Hispanic, and Asian donor samples should all be included in studies supporting licensure but are not required on each lot. All

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methods listed in the manufacturer's labeling also should be studied initially. See field trials PTC for further information.

3. Antibody stability

Each lot of antibody should be monitored for potency at appropriate intervals. Once a firm has sufficient experience and data to demonstrate consistent production of a stable product, and experience with the marketed final products supports the finding of adequate stability, another protocol for testing representative lots (e.g., $\sqrt{n} + 1/\text{yr}$, where "n" equals the number of lots of product produced per year) may also be found satisfactory.

NOTE: The check on lot stability should not be confused with the more demanding protocol for demonstrating stability of a new product.

4. Microbial Contamination

Appropriate tests to demonstrate that the product meets labeling claims should be performed. The absence of mycoplasma should be demonstrated in raw materials and the process and facility validation rather than in tests on each lot.

5. Miscellaneous

Tests to confirm that the product is within the ranges specified by the license application for pH, protein concentration, ionic strength, and any other critical parameters should be performed on each lot. The concentration of additives to retard microbial growth may be calculated rather than measured.

[DOCKET NO. 84S-0182]

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RECOMMENDED METHODS FOR
EVALUATING POTENCY, SPECIFICITY, AND REACTIVITY
OF ANTI-HUMAN GLOBULIN

March 1992

For further information about this draft, contact:

Center for Biologics Evaluation and Research (HFB-940)
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD 20892
301-227-6487.

Submit written comments on this draft to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
Rm. 1-23
12420 Parklawn Drive
Rockville, MD 20857.

Submit requests* for single copies of this draft to:

Congressional, Consumer, and International Affairs Branch (HFB-142)
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
301-295-8228
FAX 301-295-8266.

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Congressional, Consumer, and International Affairs Staff (HFB-142)
Food and Drug Administration
Suite 109, Metro Park North 3
7364 Standish Place
Rockville, MD 20855.

Comments and requests should be identified with the docket number found in brackets in the heading of this document.

PROPOSED REVISION

RECOMMENDED METHODS FOR
ANTI-HUMAN GLOBULIN EVALUATION

MARCH 1992

In follow-up to the 7-9 November 1990 workshop "REAGENTS FOR THE 1990's", the comments received from licensed manufacturers of Blood Grouping Reagents, users, and other interested parties concerning "Proposed Revised Performance Criteria for Anti-Human Globulin Reagents" were reviewed and incorporated into this document. A summary of the changes that have been made is included on page (ii).

Subsequent to the publication of the Federal Register Notice of Availability, all licensed manufacturers and other interested parties will have the opportunity to review and comment on the draft.

Specific references to the pages which your comments relate to or photocopies of the pages with your comments written on them would be helpful and appreciated. Responses are requested within 60 days of the date of publication of Federal Register Notice of Availability, however, comments are welcome at any time.

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

PROPOSED REVISION

RECOMMENDED METHODS FOR
ANTI-HUMAN GLOBULIN EVALUATION

MARCH 1992

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SUMMARY OF COMMENTS INCORPORATED AND CHANGES MADE

BASED ON WORKSHOP DISCUSSIONS CONCERNING
"PROPOSED REVISED PERFORMANCE CRITERIA FOR ANTI-HUMAN GLOBULIN REAGENTS"

1. Correction and clarification of centrifugation times and speeds.
2. The potency tests against cells sensitized with anti-D and anti-Fy* were revised to limit the number of tubes being read at a given time.
3. The warm low ionic methods of coating red blood cells with C3b/C4b and C3d/C4d have been included as alternative methods for complement coating red blood cells.
4. The test against enzyme treated cells has been modified to allow the use of papain and/or ficin.
5. Typographical errors were corrected.

SUMMARY OF COMMENTS RECEIVED BUT NOT INCORPORATED

1. The suggested changes to terminology, i.e. to change C3b to C3c and C4b to C4c, were not made because the workshop indicated there was incomplete agreement on this issue. FDA would appreciate additional consideration of this point because common terminology for the future would certainly be an asset and it is hoped that current revision efforts will serve international standardization objectives.
2. Several people suggested deleting some of the control cells instituted in the 1st draft. Those deletions have not been made because it is believed they are valid and necessary controls. Additionally, the various suggestions for deletion of control cells were not in agreement.
3. The suggestion to include a test to detect prozones needs further definition from the manufacturers.
4. The suggestion to include a test for resistance to inhibition also requires additional data to ensure effectiveness before general use.

NOTE: Suggested methods for both items 3 and 4 above will be incorporated and voluntary performance encouraged. Licensed manufacturers are requested to perform these tests for prozone and inhibition on 10 lots of product within the next 12 months and submit data to FDA for evaluation. Manufacturers who do routinely test each lot and obtain satisfactory results may so state in package insert labeling.

RECOMMENDED METHODS FOR ANTI-HUMAN GLOBULIN EVALUATION

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

- A. The Reference Anti-Human Globulin preparations listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-IgG

Anti-C3d

NOTE: FDA Reference Anti-Human Globulin preparations are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

- B. Reference Anti-Human Globulin preparations are to be used according to the accompanying package insert only for determining the potency of anti-IgG and anti-C3d as part of their final lot release testing. These reference preparations should be run in parallel with the reagent under test.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. GENERAL CONSIDERATIONS

A. RED BLOOD CELLS

1. Red blood cells for use in control testing should be used within seven days of collection or prepared from red blood cells frozen within seven days of collection. Blood samples should be collected in approved anticoagulants.
2. Red blood cells should be washed at least twice with isotonic saline or until a clear supernate is obtained.
3. Throughout these methods the diluent for cell suspensions should be isotonic saline containing 1-2% bovine albumin (unless otherwise specified) and the concentration of red blood cell suspensions should be 2%.
4. Uncoated red blood cells should give negative direct antiglobulin tests with polyspecific Anti-Human Globulin reagents.
5. Red blood cells coated with immunoglobulin or complement by one of the methods detailed herein may be frozen and thawed for use in potency and specificity testing, unless otherwise noted.

Frozen red blood cells should be used on the day of thawing unless data demonstrating that longer storage is effective has been accepted by the FDA. To ensure that the correct sample was thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells.

The method of freezing, storing and thawing red blood cells, including a description of the cryoprotective medium, should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research, before use in control testing of licensed products.

B. SEROLOGIC CONTROLS

1. Red blood cells coated with complement or immunoglobulin should be tested each day of use with the following positive and negative control reagents to assure reactivity.

CELLS WITH	POSITIVE CONTROLS	NEGATIVE CONTROLS
C3b	Anti-C3b or Anti-C3b,-C3d	Anti-C4b,-C4d; Anti-IgG
C3d	Anti-C3d	Anti-C3b; Anti-C4b,-C4d; Anti-IgG
C4b	Anti-C4b	Anti-C3b,-C3d; Anti-IgG
C4d	Anti-C4d or Anti-C4b,-C4d	Anti-C4b; Anti-C3b,-C3d; Anti-IgG
IgA	Anti-IgA	Anti-C3b,-C3d; Anti-C4b,- C4d; Anti-IgG (Heavy Chain Specific)
IgG	Anti-IgG (Heavy Chain Spec.)	Anti-C3b,-C3d; Anti-C4b,- C4d; Anti-IgA

When specific control reagents are unavailable, approval to use alternative procedures may be obtained from the Director, Center for Biologics Evaluation and Research.

For the purpose of these methods, the Center for Biologics Evaluation and Research has defined the antibody which reacts only with C3b coated red blood cells as anti-C3b. It is recognized that the antibody produced in response to immunization is usually directed against the antigenic determinant A which is located on the C3c subunit; some have called this antibody "anti-C3c". C3b coated red blood cells prepared by the Fruitstone method will react with Anti-C3d reagents. These red blood cells may also be coated with trace amounts of IgG.

Red blood cells coated with IgA myeloma proteins may also be coated with IgG. Therefore, to avoid misleading test results, the anti-IgG component of Anti-Human Globulin may be neutralized by adding an equal volume of Rh₀(D) Immune Globulin (Human) diluted 1:1000.

C. REAGENT DILUTIONS

1. The diluent for reagent dilutions should be 1-2% bovine albumin in isotonic saline.
2. Master, serial, two-fold dilutions for potency titrations should be made in tubes large enough to facilitate adequate mixing of the contents (i.e. 12 X 75 mm or larger tubes).
3. A separate clean pipette or plastic tip should be used for each dilution to avoid carryover.
4. The last tube should contain diluent only and serve as a diluent control.

D. CENTRIFUGATION

1. Unless otherwise specified, all tests are centrifuged for 15 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

E. REACTION GRADING

1. Cell buttons should be gently dislodged from the tube and observed macroscopically. The reactions should be graded and recorded as follows:
 - 4+ cell button remains in one clump
 - 3+ cell button dislodges into several clumps
 - 2+ cell button dislodges into many small clumps of equal size
 - 1+ cell button dislodges into finely granular but definitely small clumps
 - D cell button dislodges into fine granules but not definite small clumps. Results should be recorded as doubtful. For the purpose of potency testing, doubtful reactions are deemed to be negative.
 - 0 Negative - cell button dislodges with no visible clumps.

III. POTENCY TEST METHODS

A. TEST PROCEDURE FOR DETERMINATION OF ANTI-IgG USING RED BLOOD CELLS COATED WITH ANTI-D.

1. Select an anti-D serum for coating test red blood cells that meets the criteria listed below.

The undiluted anti-D should not directly agglutinate a 2% suspension of Group O, Dce or DCce (R_r or R_r) red blood cells. If undiluted single donor serum is not available, approval for the use of pooled or single donor antisera which are diluted may be requested from the Director, Center for Biologics Evaluation and Research. Monoclonal anti-D should not be used.

The anti-D serum used for cell coating should have a 1+ titration endpoint no less than 16 and no greater than 64 by the following test procedure.

- a. Beginning with undiluted anti-D serum, prepare separate master, serial, two-fold dilutions (1:2, 1:4 etc.) of the serum.
- b. To 0.1 ml of each anti-D serum dilution add 0.1 ml of a 2% suspension of Dce or DCce (R_r or R_r) red blood cells.
- c. Mix and incubate at 37°C for 30 minutes.
- d. Wash four times with large volumes of isotonic saline.
- e. To each cell button add 0.1 ml of a released lot of licensed Anti-Human Globulin containing anti-IgG. Mix.
- f. Centrifuge and record reactions.

Potency testing of the anti-D serum need not be performed each day of test provided that the same lot of serum and the same red cell donor source are used to determine the anti-IgG potency of the Anti-Human Globulin reagent under test.

2. Coat the red blood cells with anti-D as follows.
 - a. Beginning with the anti-D serum or an approved dilution of the serum meeting the criteria for cell coating (III.A.1), prepare master, serial, two-fold dilutions (1:2, 1:4, etc.) of

this serum. Include at least two dilutions of anti-D immediately following the endpoint that was observed when selecting the serum.

- b. Add to the dilutions of anti-D an equal volume of the 2% red cell suspension used in selecting the anti-D serum and mix thoroughly.
 - c. Incubate at 37°C for 30 minutes.
 - d. After incubation, wash the coated red blood cells four times with large excesses of isotonic saline and resuspend to the original 2% red cell concentration.
3. Prepare dilutions of the Anti-Human Globulin reagent under test as well as the Reference Anti-IgG preparation as follows.

Beginning with undiluted reagent, prepare separate master, serial, two-fold dilutions (undiluted through 1:8) of the Anti-Human Globulin under test and the Anti-IgG reference preparation.

4. Perform the test for potency as follows.
 - a. For both the reagent under test and the Reference Anti-IgG preparation, prepare at least four sets of test tubes, each containing no fewer than seven tubes. Each set should represent a dilution of the Anti-Human Globulin as prepared in III.A.3. To each tube of the first set place 0.1 ml of undiluted Anti-Human Globulin; to each tube of the second set place 0.1 ml of Anti-Human Globulin diluted 1:2, etc.
 - b. To the last tube of each set add 0.1 ml of red blood cells which are not coated with anti-D. Mix, centrifuge and record reactions observed.
 - c. To the second to the last tube of each set, add 0.1 ml of red blood cells coated with the highest dilution of the anti-D. Mix, centrifuge and record reactions observed.
 - d. Continue the serial addition of red blood cells coated with increasing amounts of anti-D until all sets are completed.

B. TEST PROCEDURE FOR DETERMINATION OF ANTI-IgG USING RED BLOOD CELLS COATED WITH ANTI-Fy^a

1. Select an anti-Fy^a for coating test red blood cells that meets the criteria listed below.

Antibodies from pools of donors that have been deliberately hyperimmunized to produce reagent quality antiserum may not be suitable because they may not demonstrate the variations in reactivity that are sometimes seen with weaker antibodies observed in patient samples. However, the Center for Biologics Evaluation and Research will consider approval of pooled or single donor antisera which are diluted if undiluted single donor antisera are not readily available.

The anti-Fy^a used for coating red blood cells should have a 1+ titration endpoint of no less than 8 and no greater than 32 using a 2% suspension of group O, Fy(a+b+) red blood cells by the following procedure.

- a. To 0.5 ml of anti-Fy^a add 0.07 ml of 0.11 M (4.45%) dipotassium ethylenediamine-tetraacetic acid (K₂EDTA). Incubate the serum-EDTA mixture at room temperature (20°C to 30°C) for 15 to 20 minutes.
- b. Beginning with undiluted anti-Fy^a serum, prepare separate master, serial, two-fold dilutions (1:2, 1:4, etc.) of the serum.
- c. To 0.1 ml of each anti-Fy^a serum dilution add 0.1 ml of the 2% suspension of Fy(a+b+) red blood cells.
- d. Mix and incubate at 37°C for 30 minutes.
- e. Wash four times with large volumes of isotonic saline.
- f. To each cell button add 0.1 ml of a released lot of licensed Anti-Human Globulin containing anti-IgG. Mix.
- g. Centrifuge and record reactions observed.

Potency testing of the anti-Fy^a serum need not be performed each day of test provided that the same lot of serum and the same red cell donor source are

used to determine the anti-IgG potency of the Anti-Human Globulin reagent under test.

2. Coat the red blood cells with anti-Fy^a as follows.
 - a. To three ml of anti-Fy^a serum or approved serum dilution meeting the criteria for cell coating (III.B.1), add 0.38 ml of 0.11 M (4.45%) K₂EDTA. Incubate the serum-EDTA mixture at room temperature (20°C to 30°C) 15 to 20 minutes.
 - b. Using the anti-Fy^a serum-EDTA mixture, prepare master, two-fold, serial dilutions (undiluted, 1:2, 1:4, etc.). Include at least two dilutions immediately following the end point observed when selecting the anti-Fy^a serum in III.B.1.
 - c. Add to the dilutions of anti-Fy^a an equal volume of the red cell suspension used in selecting the anti-Fy^a serum.
 - d. Incubate at 37°C for 30 minutes.
 - e. After incubation, wash the coated red blood cells four times with large excesses of isotonic saline and resuspend to the original 2% red cell concentration.
3. Prepare dilutions of the Anti-Human Globulin under test as well as the Reference Anti-IgG preparation as follows.

Beginning with undiluted reagent, prepare separate master, serial, two-fold dilutions (undiluted through 1:8) of the Anti-Human Globulin under test and the Anti-IgG Reference preparation.
4. Perform the test for potency as follows.
 - a. For both the reagent under test and the Reference Anti-IgG preparation, prepare at least four sets of test tubes, each containing no fewer than six tubes. Each set should represent a dilution of the Anti-Human Globulin as prepared in III.B.3. To each tube of the first set, place 0.1 ml of undiluted Anti-Human Globulin; to each tube of the second set, place 0.1 ml of Anti-Human Globulin diluted 1:2, etc.

- b. To the last tube of each set add 0.1 ml of red blood cells which are not coated with anti-Fy^a Mix, centrifuge and record reactions observed.
- c. To the second to the last tube of each set, add 0.1 ml of red blood cells coated with the highest dilution of the anti-Fy^a Mix, centrifuge and record reactions observed.
- d. Continue the serial addition of red blood cells coated with increasing amounts of anti-Fy^a until all sets are completed.

C. TEST PROCEDURE FOR DETERMINATION OF ANTI-C3b.^{1,2,6}

Please note that the amount of complement coated to the red blood cells *in vitro* may vary from donor to donor. It is recommended that initial testing include at least five donors to determine the best donor for use. Each donors' red blood cells should be sensitized with its own plasma or serum.

1. Solution J and Solution H are needed for this procedure. (See the appendix of Stock Solutions).
2. Prepare red blood cells coated with C3b as follows.

At least two normal clotted or anticoagulated whole blood samples should be collected and treated within one hour of collection as follows:

- a. Place 19.8 ml of Solution J in a 0°C ice bath and stir gently with magnetic stirring bar.
- b. Add 0.5 ml of washed, packed group O red blood cells.
- c. Add 0.5 ml of the same donor's fresh serum or plasma diluted 1:50 with isotonic saline.
- d. Immediately add 0.1 ml of Solution H to each of the above mixtures.
- e. Incubate for 30 minutes at 0°C with constant, gentle, stirring. Monitor the temperature to ensure 0°C is maintained.
- f. Wash the red blood cells four times with large excesses of isotonic saline and remove supernatant from the packed red blood cells.
- g. Resuspend each preparation to a 2% suspension.

- h. Store at 4°C. Use within three days or aliquot and freeze immediately.
3. Prepare "sucrose control" red blood cells for each donor in the same manner but substitute isotonic saline for the diluted serum or plasma in step III.C.2.c.
4. Prepare dilutions of the Anti-Human Globulin under test as follows.

Beginning with undiluted reagent, prepare master, serial, two-fold dilutions (1:2, 1:4, through 1:8) of the Anti-Human Globulin under test.
5. Perform the test for potency as follows.
 - a. Place 0.1 ml of each dilution of the test reagent into a separate test tube for each donor of the prepared C3b coated test cell suspensions.
 - b. Add 0.1 ml from each of the C3b coated test cell suspensions to each test dilution of Anti-Human Globulin.
 - c. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - d. Centrifuge and record reactions observed.
6. Perform a "sucrose control" test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of the "sucrose control" cell suspension prepared with the C3b coated red blood cells.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
7. Perform a negative control test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of untreated red blood cells from the same donors as in III.C.2 and III.C.3.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.

c. Centrifuge and record reactions observed.

D. TEST PROCEDURE FOR DETERMINATION OF ANTI-C3d.

1. Solution S is needed for this procedure. (See the appendix of Stock Solutions).
2. Prepare the C3d coated red blood cells as follows.

These red blood cells should be prepared from the same blood sample used to prepare the C3b test red blood cells.

- a. Prepare C3b coated packed red blood cells from two blood samples as previously outlined in III.C.
 - b. Place 0.5 ml of each of the C3b coated red blood cells in a test tube.
 - c. Add 1.0 ml of Solution S to each of the tubes.
 - d. Mix and incubate at 37° C for 30 minutes with occasional further mixing.
 - e. Wash the red blood cells four times with large excesses of isotonic saline and resuspend to a 2% concentration.
 - f. Store at 4°C. Use within three days or aliquot and freeze immediately.
3. Prepare "trypsin control" red blood cells for each donor in the same manner but substitute the "sucrose control" red blood cells from step III.C.3 for the washed, packed red blood cells in III.D.2.b.
 4. Dilute the Anti-Human Globulin under test and the Reference Anti-C3d preparation as follows.

Beginning with undiluted reagent, prepare master, serial, two-fold dilutions (1:2, 1:4, through 1:8) of the Anti-Human Globulin under test and the Reference Anti-C3d preparation.
 5. Perform the test for potency as follows.
 - a. Place 0.1 ml of each dilution of the Anti-Human Globulin reagent under test and the Reference Anti-C3d preparation into a separate

test tube for each donor of the C3d coated test cell suspensions.

- b. Add 0.1 ml from each of the C3d coated test cell suspensions to each test dilution of Anti-Human Globulin.
 - c. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - d. Centrifuge and record reactions observed.
6. Perform a "trypsin control" test as follows.
- a. To 0.1 ml of the undiluted Anti-Human Globulin add 0.1 ml of the "trypsin control" cell suspension prepared with the C3d coated red blood cells.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
7. Perform a negative control test as follows.
- a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of untreated red blood cells from the same donors as in III.C.2 and III.C.3.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.

E. TEST PROCEDURE FOR DETERMINATION OF ANTI-C4b.^{4,6}

- 1. Solution K and Solution N are needed for this procedure. (See the appendix of Stock Solutions.)
- 2. Prepare the C4b coated test red blood cells as follows.

At least two normal clotted blood samples should be collected and treated within one hour of collection as follows:

- a. Place 10 ml of Solution K in a large tube.
- b. Add 0.15 ml of Solution N.

- c. Add 0.5 ml of washed, packed group O red blood cells.
 - d. Add 0.5 ml of fresh serum.
 - e. Mix well and incubate at 37°C for 15 minutes.
 - f. Wash the red blood cells four times with isotonic saline.
 - g. Resuspend the red blood cells to a 2% concentration.
 - h. Store at 4°C. These red blood cells should be used within 3 days or aliquot and freeze immediately.
3. Prepare "sucrose control" red blood cells for each donor in the same manner but substitute isotonic saline for the fresh serum in step III.E.2.d.
 4. Dilute the Anti-Human Globulin under test as follows.
 - a. Beginning with undiluted reagent, prepare master, serial, two-fold dilutions (1:2, 1:4 through 1:8) of the reagent under test.
 5. Perform the test for potency as follows.
 - a. To 0.1 ml of each Anti-Human Globulin dilution, add 0.1 ml from each of the C4b coated test cell suspensions.
 - b. Mix, thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
 6. Perform a "sucrose control" test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin add 0.1 ml of the "sucrose control" cell suspension prepared with the C4b coated red blood cells.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.

7. Perform a negative control test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of untreated red blood cells from the same donors as in III.E.2 and III.E.3.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
- F. TEST PROCEDURE FOR DETERMINATION OF ANTI-C4d.⁴⁴
 1. Solution S is needed for this procedure. (See the appendix of Stock Solutions).
 2. Prepare the C4d coated red blood cells as follows.

These red blood cells should be prepared from the same blood sample used to prepare the C4b test red blood cells.

 - a. Prepare C4b coated packed red blood cells from two blood samples as previously outlined in III.E.
 - b. Place 0.5 ml of each of the C4b coated red blood cells in a test tube.
 - c. Add 1.0 ml of Solution S to each of the tubes.
 - d. Mix and incubate at 37°C for 30 minutes with occasional further mixing.
 - e. Wash the red blood cells four times with large excesses of isotonic saline and resuspend to a 2% concentration.
 - f. Store at 4°C. Use within three days or aliquot and freeze immediately.
 3. Prepare "trypsin control" red blood cells for each donor in the same manner but substitute the "sucrose control" red blood cells from step III.E.3 for the washed, packed red blood cells in III.E.2.c.

4. Dilute the Anti-Human Globulin under test as follows.
 - a. Beginning with undiluted reagent prepare master, serial, two-fold dilutions (1:2, 1:4, through 1:8) of the reagent under test.
 5. Perform the test for potency as follows.
 - a. To 0.1 ml of each dilution of the test reagent, add 0.1 ml of the C4d coated cell suspensions.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
 6. Perform a "trypsin control" test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin add 0.1 ml of the "trypsin control" cell suspension prepared in III.F.2.c. above.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
 7. Perform a negative control test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of untreated red blood cells from the same donors as in III.E.2 and III.E.3.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.
- G. TEST PROCEDURE FOR DETERMINATION OF ANTI-C3b/C4b⁶
1. Solution A and Solution B are needed for this procedure. (See the appendix of Stock Solutions).
 - a. Prepare Solution A1 by diluting 5.0 ml of Solution A to 1000 ml with DI water.
 - b. Prepare Solution B1 by diluting 5.0 ml of Solution B to 1000 ml with DI water.

- c. Place Solution A1 in a 1000 ml beaker and add Solution B1 until pH 6.1 is achieved.
 - d. Weigh out 92.4 gm of sucrose and dissolve in and QS to 1000 ml with the buffer prepared in G.1.c. above. (This solution may be aliquoted and stored at -20° C or below.)
2. Prepare red blood cells coated with C3b/C4b as follows.

At least two normal clotted whole blood samples should be collected and treated within 18 hours of collection as follows:

- a. Place 8.5 ml of the buffer from III.G.1.d. into a 20 - 25 ml container.
 - b. Add 0.5 ml of fresh group O serum and mix.
 - c. Immediately add 1.0 ml of washed group O cells (from the same donor) at a concentration of 50% in isotonic saline.
 - d. Mix and incubate at 37°C for 30 minutes with occasional further mixing.
 - e. Centrifuge the red blood cells and discard the supernatant. Wash the red blood cells 4 times with large excesses of isotonic saline, discarding the last wash.
 - f. Resuspend each preparation to a 2% suspension.
 - g. Store at 4° C. Use on the day of preparation only.
3. Prepare "sucrose control" red blood cells for each donor in the same manner but substitute isotonic saline for the serum in step III.G.2.b.
 4. Prepare "negative control" red blood cells by making a 2% suspension of untreated red blood cells.
 5. These coated red blood cells and their control red blood cells may be used in the same manner as those prepared in III.C. and III.E.

H. TEST PROCEDURE FOR DETERMINATION OF ANTI-C3d/C4d:

1. Solution S is needed for this procedure. (See the appendix of Stock Solutions).
2. Prepare C3d/C4d coated red blood cells as follows.

These red blood cells should be prepared from the same blood sample used to prepare the C3b/C4b test red blood cells.

 - a. Prepare C3b/C4b coated packed red blood cells from the two blood samples as previously outlined in III.G.
 - b. In a 20 - 25 ml container, mix 1.0 ml of Solution S and 0.5 ml of washed, packed C3b/C4b red cells.
 - c. Incubate at 37°C for 30 minutes with occasional further mixing.
 - d. Centrifuge the red blood cells and discard the supernatant. Wash the red blood cells 4 times with large excesses of isotonic saline, discarding the last wash.
 - e. Resuspend each preparation to a 2% suspension.
 - f. Store at 4° C. Use on the day of preparation only.
3. Prepare "trypsin control" red blood cells for each donor in the same manner but substitute "sucrose control" red blood cells for the C3b/C4b cells in step III.H.2.b.
4. Prepare "negative control" red blood cells by making a 2% suspension of untreated red blood cells.
5. These coated red blood cells and their control red blood cells may be used in the same manner as those prepared in III.D. and III.F.

I. TEST PROCEDURE FOR IgA DETERMINATION⁵.

1. Prepare the following reagents.
 - a. 1% Chromic Chloride Stock Solution (store in dark bottle at 4°C). Dilute 1:20 in unbuffered isotonic saline for use.

- b. Purified IgA myeloma protein (0.1 mg/ml in unbuffered isotonic saline).
2. Prepare the IgA coated red blood cells as follows.

Obtain group O red blood red blood cells from at least two donors and wash them four times with unbuffered saline.

 - a. Place 0.1 ml of washed packed red blood cells in a test tube.
 - b. Add 0.1 ml of diluted IgA protein. Mix.
 - c. Add 0.1 ml of diluted CrCl₃ and mix immediately.
 - d. Mix continually for four minutes at room temperature.
 - e. Wash the red blood cells four times with large excesses of unbuffered isotonic saline and discard supernatant from the packed red blood cells.
 - f. Resuspend the red blood cells to a 2% concentration.
3. Prepare "chromic chloride control" red blood cells for each donor in the same manner but substitute isotonic saline for the IgA in step III.G.2.b.
4. Perform the test for potency as follows.
 - a. To 0.1 ml of each Anti-Human Globulin reagent under test add 0.1 ml from each of the IgA coated red blood cells.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record macroscopic reactions observed.
4. Perform a "chromic chloride control" test as follows.
 - a. To 0.1 ml of the undiluted Anti-IgA add 0.1 ml of a 2% suspension of the "chromic chloride control" red blood cells prepared in III.G.3.
 - b. Incubate for 5 minutes at 20°C to 30°C.

- c. Centrifuge and record reaction.
5. Perform a negative control test as follows.
 - a. To 0.1 ml of the undiluted Anti-Human Globulin under test, add 0.1 ml of untreated red blood cells from the same donors as in III.G.2.
 - b. Mix thoroughly and incubate at 20°C to 30°C for 5 minutes.
 - c. Centrifuge and record reactions observed.

J. POTENCY TEST VALUES

The following potency values should apply to all Anti-Human Globulin specificities claimed to be present.

1. Potency titer values for the anti-IgG component.

Each dilution of the reagent tested in parallel should give reactions equal to or greater than the correspondingly diluted Reference Anti-Human Globulin preparation when tested against red blood cells coated with the smallest amounts of IgG detectable by the Reference Anti-Human Globulin preparation. The reagent under test should not prozone-i.e., no dilution of the reagent under test should give significantly stronger reactions than those observed with the undiluted reagent under test.

2. Potency titer values for the Anti-C3d component.

The undiluted Anti-Human Globulin should give at least a 2+ reaction with C3d coated red blood cells and the reagent should have a potency titer value at least equal to that of the Reference Anti-C3d preparation.

3. For other complement or immunoglobulin antibodies claimed, the undiluted reagent should give at least a 2+ reaction and a 1:4 dilution of the reagent should give at least a 1+ reaction with red blood cells prepared by a method approved by Center for Biologics Evaluation and Research.

IV. METHODS FOR EVALUATING SPECIFICITY

A. TESTS AGAINST IMMUNOGLOBULIN AND/OR COMPLEMENT COATED RED BLOOD CELLS

The specificity of the Anti-Human Globulin reagent under test should be evaluated by the direct antiglobulin method described in the manufacturer's package insert with red blood cells prepared by approved methods such as those described in part III.

1. The following tests are recommended.

ANTI-HUMAN GLOBULIN REAGENT	VS	CELLS COATED WITH
Anti-IgG, -C3d (polyspecific)		IgG, C3b, C3d, C4b, C4d
Anti-IgG (Heavy Chain Specific)		IgG, IgA, IgM, C3b, C3d, C4b, C4d
Anti-IgG		IgG, C3b, C3d, C4b, C4d
Anti-Complement reagents		IgG, C3b, C3d, C4b, C4d

2. Alternative protocols may be acceptable; submit a description of the alternative procedures to the Center for Biologics Evaluation and Research for approval.
3. In addition, if the labeling claims the presence or absence of anti-C4b and/or anti-C4d, the appropriate coated red blood cells should be tested.
4. If monospecific anti-C3d activity is claimed, the absence of anti-C3b activity should be confirmed by a method approved by the Center for Biologics Evaluation and Research.
5. Testing with IgA coated red blood cells is recommended if labeling claims that the product contains, or is free of, anti-IgA activity.
6. If the presence or absence of Anti-IgM activity is claimed, this reactivity should be evaluated by methods approved by the Director, Center for Biologics Evaluation and Research.

7. Tests with IgM coated red blood cells may serve to exclude the presence of antibody to light chains.

B. TEST FOR HETEROSPECIFIC ANTIBODIES

The product should be free of antibodies capable of agglutinating or hemolyzing untreated human red blood cells.

1. A 2% red cell concentration in isotonic saline containing 1% autologous serum or plasma should be prepared from blood from normal, healthy donors within seven days of collection. Group O, A, and B red blood cells should be prepared.

2. The Test

- a. Divide nine test tubes into three sets each containing three tubes.
- b. Into each tube place 2 drops (or the amount recommended in the manufacturer's package insert) of undiluted Anti-Human Globulin under test.
- c. To the first tube of each set, add one drop of the group O red cell suspension.
- d. To the second tube of each set, add one drop of the group A red cell suspension.
- e. To the third tube of each set, add one drop of group B red cell suspension. Mix all tubes.
- f. Incubate the tubes:

Set No. 1 at 37°C for 30 minutes

Set No. 2 at 2°C to 8°C for 30 minutes

Set No. 3 at 20°C to 30°C for 30 minutes

- g. Centrifuge and examine each tube for hemolysis after centrifugation. Gently dislodge the cell button, observe macroscopically and record reactions.

NOTE: If the manufacturer recommends microscopic interpretation of results then this test should be examined microscopically.

The product is satisfactory when there is no agglutination or hemolysis in any of the tubes.

If the manufacturer would prefer to perform the test for heterospecific antibodies at an earlier stage in production, he may amend his product license appropriately. The test results should be reported on the protocol whether the test is performed on final containers or in-process product.

C. TEST FOR REACTIVITY WITH NORMAL CELL SAMPLES

The product should be free of antibodies capable of agglutinating human red blood cells obtained from donor segments collected in FDA approved anticoagulants and stored at 2°C to 8°C. The age of the segments should span the approved shelf life of donor blood.

1. Cell Suspensions

- a. Obtain six group A, B and O donor segments as outlined above.
- b. Wash the red blood cells from the segments four times in isotonic saline and resuspend one portion to a 2% cell concentration in isotonic saline and another portion to a 2% cell concentration in LISS* solution.

2. Obtain six serum samples which contain no alloantibodies and were collected within 24 hours of product testing. These donors should be ABO group compatible with the six donor segments in IV.C.1.

* Refer to the manufacturer's package insert for the low ionic additive reagent being used. The recommended volumes for cells, serum and low ionic additive reagents should be followed to achieve the desired molarity.

3. Perform the test as follows.

- a. Place one drop of serum from IV.C.2 into two separate tubes for each of the six serum donors.
- b. To the first tube add one drop of a compatible donor's isotonic saline suspended red blood cells and to the second tube, add one drop of the same donor's red blood cells suspended in LISS*. Continue until red blood cells from all six donor samples have been added to the appropriate compatible serum samples. Mix.

- c. Centrifuge. Examine the supernatants for hemolysis and record macroscopic agglutination.
- d. Incubate the test for 15 minutes at 37°C. Centrifuge. Examine the supernatants for hemolysis. Record macroscopic agglutination.
- e. Wash the red blood cells four times in isotonic saline. Add 2 drops (or the amount recommended in the manufacturer's package insert) of the Anti-Human Globulin under test to the contents of each tube.
- f. Centrifuge. Examine the contents of each tube macroscopically and microscopically and record results observed.
- g. The product is satisfactory when there is no hemolysis and no agglutination visible macroscopically or microscopically in any of the tubes.

* Refer to the manufacturer's package insert for the low ionic additive reagent being used. The recommended volumes for cells, serum and low ionic additive reagents should be followed to achieve the desired molarity.

D. TEST WITH ENZYME-TREATED RED BLOOD CELLS

Anti-Human Globulin should not agglutinate enzyme treated red blood cells.

1. Obtain red blood cell samples from at least one group A, one group B, and one group O donor.
2. Enzyme treat the red blood cells as follows.
 - a. Add 0.4 ml of washed packed red blood cells to 1.6 ml of 0.1 % enzyme (trypsin, papain, or ficin).
 - b. Incubate the tubes at 37°C for 30 minutes.
 - c. Immediately wash the red blood cells four times with large excesses of isotonic saline and resuspend to a 2% suspension.

3. Perform the test as follows.
 - a. Place 2 drops (or the amount recommended in the manufacturer's package insert) of the Anti-Human Globulin under test into a tube.
 - b. Add one drop of the enzyme treated red cell suspension.
 - c. Centrifuge. Examine the contents of each tube macroscopically and microscopically and record results observed.
 - d. Incubate the tubes for 10 minutes at 20°C to 30°C, centrifuge, read and record results observed.

The product is satisfactory when there is no agglutination visible macroscopically or microscopically in any of the tubes.

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APPENDIX I

STOCK SOLUTIONS¹

FOR USE IN PREPARATION OF CELLS COATED WITH COMPLEMENT COMPONENTS

SOLUTIONS FOR PREPARATION OF C3b and C3b/C4b COATED CELLS

Solution A (1.0M Dipotassium phosphate) Store at 4°C

K ₂ HPO ₄ ·3H ₂ O	228.2 gm
DI Water	to 1000 ml

Solution B (1.0M Potassium phosphate) Store at 4°C

KH ₂ PO ₄	136.1 gm
DI Water	to 1000 ml

Solution L (0.2M Disodium EDTA)

Na ₂ EDTA·2H ₂ O	7.45 gm
DI water	to 100 ml

Solution F (buffered sucrose)

Solution A	1.25 ml
Solution L	5.25 ml
Sucrose	23.1 gm
DI Water	to 250 ml

Solution G (buffered sucrose)

Solution B	1.1 ml
Solution L	5.25 ml
Sucrose	23.1 gm
DI Water	to 250 ml

Solution H (0.4M Magnesium chloride)

MgCl ₂	38.09 gm
DI Water	to 1000 ml

Solution J (pH 5.1 buffered sucrose)

Add Solution F to Solution G until pH 5.1 is attained.

SOLUTIONS FOR PREPARATION OF C4b COATED CELLS

Solution K (10% Sucrose) Aliquot and store at -20°C or below

Sucrose	25 gm
DI Water	to 250 ml

Solution L (0.2M Disodium EDTA)

Na ₂ EDTA-2H ₂ O	7.45 gm
DI Water	to 100 ml

Solution M (0.2M Tetrasodium EDTA)

Na ₄ EDTA	8.32 gm
DI Water	to 100 ml

Solution N (0.2M Trisodium EDTA)

Solution L	100 ml
Solution M	100 ml

SOLUTIONS FOR PREPARATION OF C3d, C4d AND C3d/C4d COATED CELLS

Solution A (1.0M Dipotassium phosphate) Store at 4°C

K ₂ HPO ₄ ·3H ₂ O	228.2 gm
DI Water	to 1000 ml

Solution B (1.0M Potassium phosphate) Store at 4°C

KH ₂ PO ₄	136.1 gm
DI Water	to 1000 ml

Solution Q (1.0% Trypsin) Aliquot and store at -20°C or below

Trypsin*	1.0 gm
0.05N HCl	to 100 ml

Solution R (pH 7.7 phosphate buffer, dilute) Aliquot and store at -20°C or below

Solution A	5.0 ml
DI Water	to 50 ml

Solution B	1.0 ml
DI Water	to 10 ml

Add Diluted Solution A to Diluted Solution B to pH 7.7.

Solution S (0.1% Trypsin)

Solution Q	0.1 ml
Solution R	0.9 ml

* Trypsin, twice crystallized

[DOCKET NO. 84S-0181]

DRAFT

RECOMMENDED METHODS FOR
BLOOD GROUPING REAGENTS EVALUATION

March 1992

For further information about this draft, contact:

Center for Biologics Evaluation and Research (HFB-940)
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD 20892
301-227-6487.

Submit written comments on this draft to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
Rm. 1-23
12420 Parklawn Drive
Rockville, MD 20857.

Submit requests* for single copies of this draft to:

Congressional, Consumer, and International Affairs Branch (HFB-142)
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
301-295-8228
FAX 301-295-8266.

* except that written requests delivered by carriers other than the U.S.
Postal Service for single copies of this draft should be submitted to:

Congressional, Consumer, and International Affairs Staff (HFB-142)
Food and Drug Administration
Suite 109, Metro Park North 3
7564 Standish Place
Rockville, MD 20855.

Comments and requests should be identified with the docket number found in
brackets in the heading of this document.

PROPOSED REVISION

RECOMMENDED METHODS FOR
BLOOD GROUPING REAGENTS EVALUATION

MARCH 1992

In follow-up to the 7-9 November 1990 workshop "REAGENTS FOR THE 1990's", the comments received from licensed manufacturers of Blood Grouping Reagents, users, and other interested parties concerning "Proposed Revised Performance Criteria for Blood Grouping Reagents" were reviewed and incorporated into this document. A summary of the changes that have been made is included on pages (ii-v).

Subsequent to the publication of the Federal Register Notice of Availability, all licensed manufacturers and other interested parties will have the opportunity to review and comment on the draft.

Specific references to the pages which your comments relate to or photocopies of the pages with your comments written on them would be helpful and appreciated. Responses are requested within 60 days of the date of publication of the Federal Register Notice of Availability, however, comments are welcome at any time.

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

PROPOSED REVISION

RECOMMENDED METHODS FOR
BLOOD GROUPING REAGENTS EVALUATION

MARCH 1992

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SUMMARY OF COMMENTS INCORPORATED AND CHANGES MADE

BASED ON WORKSHOP DISCUSSIONS CONCERNING
"PROPOSED REVISED PERFORMANCE CRITERIA FOR BLOOD GROUPING REAGENTS"

GENERAL CHANGES ADOPTED

1. Correction and clarification of centrifugation times. (I.D.4.)
2. Allowance for the use of red blood cells for specificity testing after 7 days of withdrawal from the donor upon approval of the Director, Center for Biologics Evaluation and Research. (III.B.1.)
3. Allowance for cells of any age, regardless of their source, for the "Test to Confirm Reactivity with Antigen Positive Cells". (III.B.1.)
4. Allowance for licensed Reagent Red Blood Cells to be used as provided for specificity testing. (III.B.3.)
5. Further reduction of the list of antigens to be tested for specificity when the source material is a previously characterized and licensed monoclonal antibody. (III.C.2.)
6. Clarification of recommendation to cover all methods listed in the manufacturer's package insert when testing for specificity. (III.D.)

CHANGES ADOPTED - METHODS FOR ABO BLOOD GROUPING REAGENT EVALUATION

1. Deletion of reference to cord cells as necessary cells.
2. Definition of reactivities for A₁ and A₁B cells. (II.C.)
3. Reduce the number of IgG coated red blood cells in the test for spontaneous agglutination from 3 to 1. (V.B.)

CHANGES ADOPTED - METHODS FOR SLIDE AND MODIFIED TUBE RH BLOOD GROUPING REAGENT EVALUATION

1. Addition of Rh category V (as well as IV and VI) cells for testing of monoclonal anti-D reagents. (III.C.4.)
2. Clarification that the 6 D⁺ cells should be of different Rh phenotypes. (III.C.4.)
3. Inclusion of C⁺ positive cells for Anti-C. (III.C.4.)

4. Clarification of the allowable use of r''r cells for Anti-C. (III.C.4.)
5. Allowance for r''r cells for Anti-CD. (III.C.4.)
6. Replace R_r cells with R_r cells on Avidity test. (IV.C.)
7. Inclusion of 2 tests to detect prozone. (V. & VI.)

FDA is requesting that manufacturers try the two methods for detecting prozone described in sections V and VI in their own laboratories or suggest others which may be more useful in detecting prozones.

CHANGES ADOPTED - METHODS FOR LOW PROTEIN* RH BLOOD GROUPING REAGENT EVALUATION (* INCLUDING CHEMICALLY MODIFIED REAGENTS)

1. Inclusion of the allowance for manufacturers to establish potency titer values other than those recommended for reagents derived from monoclonal source material upon approval of the Director, Center for Biologics Evaluation and Research. (II.F.3.)
2. Addition of Rh category V (as well as IV and VI) cells for testing of monoclonal anti-D reagents. (III.C.4.)
3. Clarification that the 6 D⁺ cells should be of different Rh phenotypes. (III.C.4.)
4. Inclusion of C⁺ positive cells for Anti-C. (III.C.4.)
5. Clarification of the allowable use of r''r cells for Anti-C. (III.C.4.)
6. Allowance for r''r cells for Anti-CD. (III.C.4.)
7. Deletion of recommendations for testing of cord cells.
8. Replace R_r cells with R_r cells on Avidity test. (IV.C.)
9. Reduce the number of IgG coated red blood cells in the test for spontaneous agglutination from 3 to 1. (V.B.)
10. Inclusion of 2 tests to detect prozone. (VI. & VII.)

FDA is requesting that manufacturers try the two methods for detecting prozone described in sections V and VI in their own laboratories or suggest others which may be more useful in detecting prozones.

CHANGES ADOPTED - METHODS FOR RARE BLOOD GROUPING REAGENT EVALUATION

1. Return potency titer values to original values. (II.F.1.)
2. Inclusion of the allowance for manufacturers to establish potency titer values other than those recommended for reagents derived from monoclonal source material upon approval of the Director, Center for Biologics Evaluation and Research. (II.F.2)
3. Deletion of many of the cells specified on the list of additional red blood cell phenotypes to be used for specificity testing. (III.C.4.)
4. Definition of reactivities for A₁ and A₁B Le(b+) cells. (III.C.4.)

SUMMARY OF COMMENTS RECEIVED BUT NOT ADOPTED

COMMENT NOT ADOPTED - GENERAL

1. Include reaction score values in potency evaluations to achieve more meaningful and consistent results.

The method of scoring agglutination reactions as described in the AABB Technical Manual could be incorporated by a manufacturer in their SOP and/or Product License Application file if it is deemed appropriate.

COMMENTS NOT ADOPTED RELATIVE TO ABO BLOOD GROUPING REAGENTS

1. A₁ and A₁B red blood cells would be the sample of choice for monoclonal Anti-A which may react preferentially with A₁ and A₁B red blood cells.

Such a product is unlikely to be developed for commercial use; appropriate additions to the list of recommended test cells can be made on the initial characterization and submission of a new product.

2. Manufacturers should test these reagents against serum suspended cells as well as IgG coated cells to detect false positive reactions due to serum anomalies.

FDA seeks additional comment on this matter. If a simple test that will give reliable results can be developed, its inclusion in these recommended methods will be considered.

3. FDA has received some comment that finding A,B red cells that are both negative with Anti-A₁ and positive with Anti-H is very difficult. Laboratory testing will be necessary to evaluate the problem. Please submit any information you have on this topic, and if possible, address lectin vs. human vs. monoclonal.

COMMENTS NOT ADOPTED RELATIVE TO RH BLOOD GROUPING REAGENTS

1. Manufacturers should perform a test that will show the ratio of IgM to IgG antibody for anti-D reagents.

There is agreement that this may be necessary, however, the comment initially was applied to products that are blends of monoclonal IgM and polyclonal IgG. It appears now that this test may be useful for all Anti-D reagents, regardless of the source material used. It is possible that a test to detect prozones will suffice for this purpose as well. Manufacturers are asked to submit relevant data.

2. Manufacturers should test Anti-D reagents against serum suspended cells as well as IgG coated cells to detect false positive reactions due to serum anomalies.

FDA seeks additional comment on this matter. If a simple test that will give reliable results can be developed, its inclusion in these recommended methods will be considered.

3. Manufacturers should test Anti-e against e variant red blood cells like hr^a and hr^b.

Manufacturers who wish to routinely perform such testing may indicate this test is done in product labeling.

4. Delete test of Anti-E reagents against cells that are E+ cE-.

Manufacturers who choose not to perform this test may indicate that it is not done in product labeling.

5. There is some question whether the potency method in the LOW PROTEIN RH BLOOD GROUPING REAGENT section will work uniformly well for chemically modified reagents. Additional testing is necessary. Please submit any pertinent data. If the method is proven not to work, it will be revised or deleted.

ABO BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

- A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-A

Anti-B

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

- B. Reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) as in paragraph 1 of this section. For Anti-A,B and Anti-A and B prepare dilutions of each Reference Blood Grouping Reagent separately.
3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.
4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the reference reagents are within an acceptable range.
2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-A	A ₁ and 3 DIFFERENT A ₂ B *
Anti-B	B and A ₁ B
Anti-A,B	A ₁ , A ₂ **, and B
Anti-A and B	A ₁ , A ₂ **, and B

* AB cells which do not react with anti-A₁ and do react with anti-H.

** A cells which do not react with anti-A₁ and do react with anti-H.

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
2. Place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.
4. Mix the contents of each tube thoroughly. Incubate for 5 minutes at room temperature (RT; 20-30 C) and centrifuge for 1 minute at approximately 1000 rpm (100-125 rcf) or 15 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.
2. The reactions should be graded as follows:
 - 4+ Cell button remains in one clump.
 - 3+ Cell button dislodges into several clumps.
 - 2+ Cell button dislodges into many small clumps of equal size.
 - 1+ Cell button dislodges into finely granular, but definite, small clumps.
 - D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.
 - 0 Negative reaction - cell button dislodges into no visible clumps.
3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.
4. Test results should include at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. ABO Reagents should have an average potency titer value at least equal to that of the reference reagent.
2. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

- a. At least 4 different donors whose red blood cells exhibit expression of the antigen should be tested.
- b. When testing Anti-A,B and Anti-A and B reagents, the reactivity with both group A and group B red blood cells should be confirmed separately, i.e. at least four group A donors should be used to confirm the reactivity of the Anti-A component and at least four group B donors should be used to confirm the reactivity of the Anti-B component.
- c. Minimum test red blood cells recommended:

REAGENT	RED BLOOD CELLS
Anti-A	A ₁ (1) and A ₂ B (3) *
Anti-B	A ₂ B (3) and B (1)
Anti-A,B	A ₁ (2), A ₂ ** (2), B (4), at least 3 different A _x ***
Anti-A and B	A ₁ (2), A ₂ ** (2), B (4), at least 3 different A _x ***

* AB cells which do not react with anti-A₁ and do react with anti-H.

** A cells which do not react with anti-A₁ and do react with anti-H.

*** A_x cells are recommended; labeling should indicate detection of group A variants. Include examples of "strong A_x cells" and "moderate A_x cells". "Weak A_x cells" are optional.

- d. Include at least one red blood cell which does not exhibit expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P₁, D, C, E, c, e, C^v, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^a, W^a, Sd^a, and Vw.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P₁, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

- a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.
- b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

- c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.

- d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States or the country in which it is sold.

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.
2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.
2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of A₁ red blood cells may not yield a 2+ reaction with Anti-A, B and Anti-A and B but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.
4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2
5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum which is free of antibodies or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-A	A ₁ , and A ₂ B *
Anti-B	B and A ₁ B
Anti-A,B	A ₁ , A ₂ **, B, and A ₂ ***
Anti-A and B	A ₁ , A ₂ **, B, and A ₂ ***

* AB cells which do not react with anti-A₁ and do react with anti-H.

** A cells which do not react with anti-A₁ and do react with anti-H.

*** A₂ red blood cells are recommended only if the reagent is recommended for detection of weak subgroups of A by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV.A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at the end of the first half of the observation time.
2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the observation time and should be reported as greater than or less than 1 mm in diameter.

V. TEST FOR SPONTANEOUS AGGLUTINATION

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATION

1. Obtain c positive group O red blood cells from one donor.
2. Coat the red blood cells heavily with an IgG anti-c such that a 3-4+ direct antiglobulin test (DAT) is achieved and positive reactions are obtained with a high protein Rh control reagent, but negative reactions are obtained with a saline control.

The exact procedure for coating the red blood cells will depend on the specific antibody chosen for coating and its strength.

C. THE TEST

1. Test the coated cell sample according to the manufacturer's package insert.

D. INTERPRETATION

1. Blood Grouping Reagents for use by a low protein tube test method should not spontaneously agglutinate immunoglobulin coated red blood cells.
2. In the event that the reagent under test does agglutinate the coated red blood cells, an effective control test or a control reagent adequate to prevent misinterpretation of blood group results should be recommended or supplied.
3. If a control test or reagent is recommended by the manufacturer, cells for use in control testing in sections II, II, and IV should give a negative direct antiglobulin test (DAT).

SLIDE AND MODIFIED TUBE RH BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

- A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-D for evaluation of IgG products

Anti-C for rapid tube test

Anti-E for rapid tube test

Anti-c for rapid tube test

Anti-e for rapid tube test

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

- B. All reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold dilutions (1 in 2, 1 in 4, etc.) of the test reagent using 20-22% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) as in paragraph 1 of this section. For test reagents containing multiple antibodies (ex. Anti-CDE) dilutions of each of the corresponding Reference Blood Grouping Reagents should be made separately.
3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.
4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the Reference Blood Grouping Reagent(s) are within an acceptable range.

2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-D	Dce (R ₀ r)
Anti-C	dCce (r'r)
Anti-E	dcEe (r"r)
Anti-c	DCcEe (R ₁ R ₂)
Anti-e	dcEe (r"r)
Anti-CD	dCce and Dce (r'r and R ₀ r)
Anti-DE	Dce and dcEe (R ₀ r and r"r)
Anti-CDE	dCce and Dce and dcEe (r'r and R ₀ r and r"r)

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
2. Place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.
4. Mix the contents of each tube thoroughly and incubate the test tubes for 15 minutes at 37 C.
5. Centrifuge for 2 minutes at approximately 1000 rpm (100-125 rcf) or 45 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.
2. The reactions should be graded as follows:
 - 4+ Cell button remains in one clump.
 - 3+ Cell button dislodges into several clumps.
 - 2+ Cell button dislodges into many small clumps of equal size.
 - 1+ Cell button dislodges into finely granular, but definite, small clumps.
 - D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.
 - 0 Negative reaction- cell button dislodges into no visible clumps.
3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Slide and Modified Tube Rh Blood Grouping Reagents should have an average potency titer value at least equal to that of the reference reagent.
2. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) Licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to

demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

- a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.
- b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different red blood cells possessing only one of the antigens for each different specificity.

ex. For Anti-CDE reagents, at least four donors should be used to confirm the reactivity of the Anti-C component, at least four donors should be used to confirm the reactivity of the Anti-D component, and at least four donors should be used to confirm the reactivity of the Anti-E component.

- c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.
2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P₁, D, C, E, c, e, C⁺, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M⁺, Wr⁺, and Sd⁺.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P₁, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

- a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.
- b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

- c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical

assurance of the absence of contaminating antibodies.

- d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

- a. Group A₁ and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A₁B red blood cells may be substituted for A₁ and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. PHENOTYPES RECOMMENDED FOR TESTING

As a minimum, red blood cells exhibiting the following phenotypes should be used in the specificity testing outlined in steps 1, 2, and 3 above.

MINIMUM	RED BLOOD CELLS
Anti-D	<p>DCce, Dce, dCce, and dcEe (R₁r, Ror, r'r, and r"r) A₁ dce, B dce, and O dce (rr) Vw positive</p> <p>3 different dce (rr) Bg(a+) *</p> <p>6 D^u samples representing different Rh phenotypes and reactive by the Indirect Antiglobulin Test only *</p>
Anti-D (monoclonal)	Category IV, V, and VI cells
Anti-C	<p>Dce, dCce, and dcEe or dcE (R₀r, r'r, and r"r or r"r") C⁺ Ce⁻ (e.g. R₂R₂ or R₂r) ** C⁺ positive (e.g. R₁r) A₁ dce, B dce, and O dce (rr)</p>
Anti-E	<p>Dce, dCce or dCe, and dcEe (R₀r, r'r or r'r', and r"r) E⁺ CE⁻ (e.g. R₂R₁ or R₂r) A₁ dce, B dce, and O dce (rr)</p>

Anti-c	dCce and DCEe or DCE or dCE (r'r and R ₁ R ₁ or R ₁ R ₂ or r'r') A ₁ DCE, B DCE, and O DCE (R ₁ R ₁) DCCee f neg (R ₁ R ₂)
Anti-e	dcEe and DCCE or DCE or dCE (r''r and R ₂ R ₂ or R ₂ R ₂ or r'r') A ₁ DCE, B DCE, and O DCE (R ₂ R ₂) DCCee f neg (R ₁ R ₂)
Anti-CD	Dce, dCce, and dcE or dcEe (R ₀ r, r'r, and r''r or r''r) A ₁ dce, B dce, and O dce (rr) r'r or r''r ***
Anti-DE	Dce, dCce or dCE, and dcEe (R ₀ r, r'r or r'r', and r''r) A ₁ dce, B dce, and O dce (rr)
Anti-CDE	Dce, dCce, and dcEe (R ₀ r, r'r, and r''r) A ₁ dce, B dce, and O dce (rr) r'r ***

- * For Anti-D reagents recommended for D⁺ testing
- ** r'r cells may be used in addition to but not as a substitute for C+ Ce- cells
- *** Recommended if labeling indicates detection of G antigen

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.
2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.
2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of C+ Ce- red blood cells may not yield a 2+ reaction with Anti-C but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.
4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2
5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Tests with group A₁ and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.
7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-D	Dce (R ₁ r)
Anti-C	dcCce (r'r) C+ Ce- (R ₂ R ₂ or R ₂ r) C ⁺ positive (R ₁ r)
Anti-E	dcEe (r''r) E+ cE- (R ₂ R ₁ or R ₂ r)
Anti-c	dcCce (r'r)
Anti-e	dcEe (r''r)
Anti-CD	Dce and dcCce (R ₂ r and r'r) r ^c r or r''r *
Anti-DE	Dce and dcEe (R ₂ r and r''r)
Anti-CDE	Dce, dcCce, and dcEe (R ₂ r, r'r, and r''r) r ^c r *

* Only if the reagent is recommended for detection of the G antigen by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the recommended observation time.
2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the recommended observation time and should be reported as greater than or less than 1 mm.

V. TEST FOR PROZONE

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

1. Obtain at least three red blood cell samples representing three different Rh phenotypes which exhibit heterozygous or weak expression of the antigen corresponding with the reagent antibody.
2. Fresh or frozen red blood cells may be used under the following conditions:
 - a. Licensed reagent red blood cells may be used any time before their expiration date.
 - b. Frozen red blood cells should have been frozen within 7 days of collection from the donor and should be used on the day of thawing.
 - c. All other red blood cell samples should be used within 7 days of collection from the donor.
3. Red blood cells should not be coated with complement or immunoglobulin (should be direct antiglobulin test negative).
4. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear

supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

REAGENT	RED BLOOD CELLS
Anti-D	DCce (R ₁ r)
Anti-C	DCcEe (R ₁ R ₂)
Anti-E	DCcEe (R ₁ R ₂)
Anti-c	DCce and DCcEe (R ₁ r and R ₁ R ₂)
Anti-e	DcEe and DCcEe (R ₂ r and R ₁ R ₂)

D. THE TEST

- For each cell sample to be tested label three tubes, "15 minutes", "30 minutes", and "60 minutes" respectively.
- Add the appropriate amount of the reagent under test to all tubes.

If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.
- Add 1 drop of each cell sample to its respective tubes.
- Mix and incubate for the time indicated on the tube label according to the manufacturer's package insert, i.e. at the temperature recommended for those tests giving a negative result.
- Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades are the same or increase as the incubation time increases, no prozone is present.
2. If the reaction grades decrease as the incubation time increases, a prozone is present.

F. RESULTS

1. At least a 2+ reaction should be obtained with ALL samples at ALL incubation times.

VI. TEST TO DETECT PROZONES - METHOD 2

A. REAGENT DILUTIONS

1. Prepare a 1+5 dilution of the reagent under test in inert human serum (group AB or compatible with the cells to be tested).

B. RED BLOOD CELL PREPARATIONS

1. See V.B.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

1. See V.C.

D. THE TEST

The 1+5 dilution and undiluted reagent will be tested in parallel.

1. For each cell to be tested, label two sets of tubes, "I.S.", "1 minute", "3 minutes", "5 minutes", and "10 minutes".
2. Add the appropriate amount of the reagent under test to all tubes.

If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.
4. Mix and incubate at room temperature for the time indicated on the tube label.

5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.
- E. INTERPRETATION OF THE TEST
1. If the reaction grades with the diluted reagent are stronger than the reaction grades with the undiluted reagent, a prozone is present.
 2. If the reaction grades with the diluted reagent are equal to or weaker than the reaction grades with the undiluted reagent, no prozone is present.
- F. RESULTS
1. The reagent should not exhibit any prozone.

LOW PROTEIN RH BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

- A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-CD for evaluation of IgM, Anti-D
products

Anti-C for saline tube test

Anti-E for saline tube test

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

For Blood Grouping Reagents for which there is no Reference Blood Grouping Reagent, it is strongly recommended that a previously approved lot of reagent be used as an in-house control reagent.

- B. All reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) or in-house control reagent as in paragraph 1 of this section. For test reagents containing multiple antibodies (ex. Anti-CDE), dilutions of each of the corresponding Reference Blood Grouping Reagents should be made separately.
3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.
4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the Reference Blood Grouping Reagent(s) or the in-house control reagent are within an acceptable range.
2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research as a license amendment before use in control testing.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-D	Dce (R ₀ r)
Anti-C	dCce (r'r)
Anti-E	dcEe (r"r)
Anti-c	DCcEe (R ₁ R ₂)
Anti-e	dcEe (r"r)
Anti-CD	dCce and Dce (r'r and R ₀ r)
Anti-DE	Dce and dcEe (R ₀ r and r"r)
Anti-CDE	dCce and Dce and dcEe (r'r and R ₀ r and r"r)

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
2. If a Reference Blood Grouping Reagent is available, place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.
4. Mix the contents of each tube thoroughly and incubate the test tubes at 37 C for 15 minutes.

If no Reference Blood Grouping Reagent is available, incubate at 37 C for the shortest period of time recommended in the manufacturer's package insert.
5. Centrifuge for 1 minute at approximately 1000 rpm (100-125 rcf) or 15 seconds at approximately 3400

rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

In the case of reagents for which no Reference Blood Grouping Reagent is available, centrifuge for the shortest period of time at the lowest speed recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.
2. The reactions should be graded as follows:
 - 4+ Cell button remains in one clump.
 - 3+ Cell button dislodges into several clumps.
 - 2+ Cell button dislodges into many small clumps of equal size.
 - 1+ Cell button dislodges into finely granular, but definite, small clumps.
 - D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.
 - 0 Negative reaction- cell button dislodges into no visible clumps.
3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.
4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Products for which Reference Blood Grouping Reagents are available should have an average potency titer value at least equal to that of the reference reagent.

2. Products of polyclonal origin which are recommended for tube test methods for which there are no Reference Blood Grouping Reagents available should have a potency titer value of at least a 1+ reaction with a 1:4 dilution of reagent:

eg. Anti-c (saline)
Anti-e (saline)

3. Products of monoclonal origin which are recommended for tube test methods for which there are no Reference Blood Grouping Reagents available should have a potency titer value of at least a 1+ reaction with a 1:8 dilution of reagent:

eg. Anti-c (saline)
Anti-e (saline)

Manufacturers that wish to establish potency titer values other than these are to obtain approval from the Director, Center for Biologics Evaluation and Research at the time of license application.

4. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS
 - a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.

- b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different red blood cells possessing only one of the antigens for each different specificity.

ex. For Anti-CDE reagents, at least four donors should be used to confirm the reactivity of the Anti-C component, at least four donors should be used to confirm the reactivity of the Anti-D component, and at least four donors should be used to confirm the reactivity of the Anti-E component.

- c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P₁, D, C, E, c, e, C^w, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^s, Wr^a, and Sd^a.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P₁, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

- a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.

- b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

- c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.
- d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

- a. Group A₁ and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A₁B red blood cells may be substituted for A₁ and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. PHENOTYPES RECOMMENDED FOR TESTING

As a minimum, red blood cells exhibiting the following phenotypes should be used in the specificity testing outlined in steps 1, 2, and 3 above.

REAGENT	RED BLOOD CELLS
Anti-D	<p>DCce, Dce, dCce, and dcEe (R₁r, Ror, r'r, and r"r) A₁ dce, B dce, and O dce (rr) Vw positive</p> <p>3 different dce (rr) Bg(a+) cells *</p> <p>6 D⁺ samples representing different Rh phenotypes and reactive by Indirect Antiglobulin Test only *</p>
Anti-D (monoclonal)	Category IV, V, and VI cells
Anti-C	<p>Dce, dCce, and dcEe or dCe (R₁r, r'r, and r"r or r"r") C+ Ce- (ex. R₂R₂ or R₂r) ** C⁺ positive (ex. R₁r) A₁ dce, B dce, and O dce (rr)</p>
Anti-E	<p>Dce, dCce or dCe, and dcEe (R₁r, r'r or r'r', and r"r) E+ CE- (ex. R₂R₂ or R₂r) A₁ dce, B dce, and O dce (rr)</p>
Anti-c	<p>dCce and DCee or DCE or dCE (r'r and R₂R₂ or R₂R₂ or r'r') A₁ DCE, B DCE, and O DCE (R₂R₂) DCcEe f neg (R₂R₂)</p>
Anti-e	<p>dcEe and DCcE or DCE or dCE (r"r and R₂R₂ or R₂R₂ or r'r') A₁ DcE, B DcE, and O DcE (R₂R₂) DCcEe f neg (R₂R₂)</p>
Anti-CD	<p>Dce, dCce, and dcE or dcEe (R₁r, r'r, and r"r" or r"r) A₁ dce, B dce, and O dce (rr) r'r or r"r ***</p>
Anti-DE	<p>Dce, dCce or dCe, and dcEe (R₁r, r'r or r'r', and r"r) A₁ dce, B dce, and O dce (rr)</p>
Anti-CDE	<p>Dce, dCce, and dcEe (R₁r, r'r, and r"r) A₁ dce, B dce, and O dce (rr) r'r ***</p>

- * For Anti-D reagents recommended for D⁺ testing.
- ** r''r cells may be used in addition to but not as a substitute for C+ Ce- cells
- *** Recommended if labeling indicates detection of G antigen

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.
2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.
2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of C+ Ce- red blood cells may not yield a 2+ reaction with Anti-C but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2
5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.
6. Tests with group A₁ and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.
7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-D	DCe ($R_i r$)
Anti-C	dCce ($r' r$) C+ Ce- ($R_i R_i$ or $R_i r$) C ⁺ positive ($R_i' r$)
Anti-E	dcEe ($r'' r$) E+ cE- ($R_i R_i$ or $R_i r$)
Anti-c	dCce ($r' r$)
Anti-e	dcEe ($r'' r$)
Anti-CD	Dce and dCce ($R_i r$ and $r' r$) $r'' r$ or $r'' r$ *
Anti-DE	Dce and dcEe ($R_i r$ and $r'' r$)
Anti-CDE	Dce, dCce, and dcEe ($R_i r$, $r' r$, and $r'' r$) $r'' r$ *

* Only if the reagent is recommended for detection of the G antigen by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the manufacturer's recommended observation time.
2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the

end of the manufacturer's recommended observation time and should be reported as greater than or less than 1 mm.

V. TEST FOR SPONTANEOUS AGGLUTINATION

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATION

1. Obtain c positive group O red blood cells from one donor. (When testing an Anti-c reagent, use Rh(D) positive group O cells.)
2. Coat the red blood cells heavily with an IgG anti-c (anti-D when testing an Anti-c reagent) such that a 3-4+ direct antiglobulin test (DAT) is achieved and positive reactions are obtained with a high protein Rh control reagent, but negative reactions are obtained with a saline control.

The exact procedure for coating the red blood cells will depend on the specific antibody chosen for coating and its strength.

C. THE TEST

1. Test the coated cell sample according to the manufacturer's package insert.

D. INTERPRETATION

1. Blood Grouping Reagents for use by the saline tube test method should not spontaneously agglutinate immunoglobulin coated red blood cells.
2. In the event that the reagent under test does agglutinate the coated red blood cells, an effective control test or a control reagent adequate to prevent misinterpretation of blood group results should be recommended or supplied.

VI. TEST FOR PROZONE

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

1. Obtain at least three red blood cell samples representing three different Rh phenotypes which exhibit heterozygous or weak expression of the antigen corresponding with the reagent antibody.
2. Fresh or frozen red blood cells may be used under the following conditions:
 - a. Licensed reagent red blood cells may be used any time before their expiration date.
 - b. Frozen red blood cells should have been frozen within 7 days of collection from the donor and should be used on the day of thawing.
 - c. All other red blood cell samples should be used within 7 days of collection from the donor.
3. Red blood cells should not be coated with complement or immunoglobulin (should be direct antiglobulin test negative).
4. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

REAGENT	RED BLOOD CELLS
Anti-D	DCce (R ₁ r)
Anti-C	DCcEe (R ₁ R ₂)
Anti-E	DCcEe (R ₁ R ₂)
Anti-c	DCce and DCcEe (R ₁ r and R ₁ R ₂)
Anti-e	DcEe and DCcEe (R ₂ r and R ₁ R ₂)

D. THE TEST

1. For each cell sample to be tested label three tubes, "15 minutes", "30 minutes", and "60 minutes" respectively.

2. Add the appropriate amount of the reagent under test to all tubes.

If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.
4. Mix and incubate for the time indicated on the tube label according to the manufacturer's package insert, i.e. at the temperature recommended for those tests giving a negative result.
5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades are the same or increase as the incubation time increases, no prozone is present.
2. If the reaction grades decrease as the incubation time increases, a prozone is present.

F. RESULTS

1. At least a 2+ reaction should be obtained with ALL samples at ALL incubation times.

VII. TEST TO DETECT PROZONES - METHOD 2

A. REAGENT DILUTIONS

1. Prepare a 1+5 dilution of the reagent under test in inert human serum (group AB or compatible with the cells to be tested.)

B. RED BLOOD CELL PREPARATIONS

1. See VI.B.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

1. See VI.C.

D. THE TEST

The 1+5 dilution and undiluted reagent will be tested in parallel.

1. For each cell to be tested, label two sets of tubes, "I.S.", "1 minute", "3 minutes", "5 minutes", and "10 minutes".
2. Add the appropriate amount of the reagent under test to all tubes.

If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.
4. Mix and incubate at room temperature for the time indicated on the tube label.
5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades with the diluted reagent are stronger than the reaction grades with the undiluted reagent, a prozone is present.
2. If the reaction grades with the diluted reagent are equal to or weaker than the reaction grades with the undiluted reagent, no prozone is present.

F. RESULTS

1. The reagent should not exhibit any prozone.

RARE BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

- A. There are no Reference Blood Grouping Reagents available for the reagents covered in this document. It is strongly recommended that a previously approved lot of reagent be used as an in-house control reagent.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the in-house control reagent as in paragraph 1 of this section.

3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.
4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the in-house control reagent are within an acceptable range.
2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research as a license amendment before use in control testing of reagent.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

1. At least 2 different donors with phenotypes exhibiting weak and/or heterozygous expression of the antigen, where applicable.

For example, Anti-Le^a and Anti-Le^b are excluded.

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).
2. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.
3. Mix the contents of each tube thoroughly and incubate the test tubes for the shortest incubation time at the temperature recommended in the manufacturer's package insert for the product. For rapid tube reagents incubate at room temperature (RT; 20-30 C) for 5 minutes.
4. Centrifuge at the lowest speed and for the shortest period of time recommended in the manufacturer's package insert.
5. Perform the indirect antiglobulin test according to the manufacturer's package insert, if recommended.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.
2. The reactions should be graded as follows:
 - 4+ Cell button remains in one clump.
 - 3+ Cell button dislodges into several clumps.
 - 2+ Cell button dislodges into many small clumps of equal size.
 - 1+ Cell button dislodges into finely granular, but definite, small clumps.
 - D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.
 - 0 Negative reaction- cell button dislodges into no visible clumps.

3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Products of polyclonal origin which are recommended for tube test methods should have an average potency titer value as follows:

- a. At least a 1+ reaction with a 1:8 dilution of reagent:

Anti-K
Anti-k
Anti-Jk^a
Anti-Fy^a
Anti-C^u

- b. At least a 1+ reaction with a 1:4 dilution of reagent:

Anti-S
Anti-s
Anti-P₁
Anti-M
Anti-I
Anti-A₁

- c. At least a 2+ reaction with undiluted reagent:

Anti-U
Anti-Kp^a
Anti-Kp^b
Anti-Js^a
Anti-Js^b
Anti-Fy^b
Anti-N
Anti-Le^a
Anti-Le^b
Anti-Di^a
Anti-M^u
Anti-Jk^b
Anti-Xg^a
Anti-Co^b
Anti-Wr^a

2. Products of monoclonal origin which are recommended for tube test methods should have an average potency titer value of at least 1+ with a 1:8 dilution of reagent.

Manufacturers that wish to establish potency titer values other than these are to obtain approval from the Director, Center for Biologics Evaluation and Research at the time of license application.

3. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.
4. Licensed reagent red blood cells may be used as provided.

All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS
 - a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.
 - b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different cells possessing only one of the antigens for each different specificity.
 - c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.
2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P₁, D, C, E, c, e, C^v, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^s, Wr^a, and Sd^a.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P₁, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

- a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.
- b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

- c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.
- d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

- a. Group A₁ and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A₂B red blood cells may be substituted for A₁ and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. ADDITIONAL PHENOTYPES RECOMMENDED FOR TESTING

REAGENT	RED BLOOD CELLS
Anti-A ₁	A ₁ , A ₂ , A ₁ B, A ₂ B, B, and O
Anti-k	K+k+ Kp(a+b+)
Anti-Le ^b	6 different A ₁ and/or A ₁ B Le(b+) *
Anti-P ₁	At least 2 P ₁ weak (as determined by titration studies)
Anti-U	S-, s-, U+
Anti-Fy ^b	Fy*
Anti-Jk ^a	Jk(a-b-)
Anti-Jk ^b	Jk(a-b-)

* Group A and AB cells which do react with anti-A₁ and do not react with anti-H.

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.
2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.
2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

When testing unusual phenotypes, other criteria for reactivity may apply. For example, Fy^a red blood cells may not yield a 2+ reaction with Anti- Fy^a but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.
4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2
5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Tests with group A₁ and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.
7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

1. Red blood cells from at least two different donors exhibiting weak and/or heterozygous expression of the antigen corresponding to the reagent antibody should be used.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the manufacturer's recommended observation time.
2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the manufacturer's recommended observation time and should be reported as greater than or less than 1 mm.

LIAISON:

AABB Standards Committee (Hoppe)

AABB Ad Hoc Committee to review Donor Screening Procedures (Hoppe)

AABB Extracorporeal Therapy Committee (Santos)

AABB Transfusion Transmitted Disease Committee (Epstein)

AABB Transfusion Practices Committee (Holness)

AABB Safety Committee (Hoppe)

AABB Technical Manual Committee (Hoppe)

AABB Autologous Transfusion Committee (Denham/Hoppe)

AABB Information Systems Committee (Hoppe/Northern)

AABB Scientific Program (Hoppe/Epstein)

AABB Technical Quality Assurance (Frantz-Bohn)

EPA Medical Waste Policy Committee (Hoppe)

Council of Europe - Committee of Experts and subcommittee on Automation and Quality Standards (Hoppe)

WHO Donor Standards Committee (Hoppe)

WHO Reagent Production Committee (Hoppe/Kochman)

ISBT Enzymes Standardization Committee (Hoppe/Kochman)

ISBT Antiglobulin Standards Committee (Hoppe/Kochman)

ISBT Monoclonal Reagents Standards Committee and Workshops (Hoppe)

NHLBI Transfusion Safety Study Policy Board (Hoppe)

NHLBI AIDS Ad Hoc Committee (Hoppe/Epstein)

National Blood Resources Education Program Policy Board (Hoppe)

A2LA (American Association of Laboratory Accreditation)-FDA Liaison to the Board of Directors (Hoppe)

Health Care Financing Administration Liaison (Hoppe)

NBREP Subcommittee on Autologous Transfusion (Hoppe)

MEMORANDUM

Date: March 17, 1992

From: Jay S. Epstein, M.D., Acting Deputy Director DTS
through Ms. P. Ann Hoppe, Acting Director, DTS

Subject: Current Issues in the Division of Transfusion Science

To: M. Carolyn Hardegree, M.D., Director, Office of Research

A. Implementation of Recently Approved Products

FDA recommendations are needed to address the implementation of recently approved products for retroviral and hepatitis testing based on policy positions which have been developed at Blood Product Advisory Committee meetings. Appropriate memoranda to blood establishments are being developed.

1. Two manufacturers have been licensed recently to distribute tests for combined screening for antibodies to HIV-1 and HIV-2, namely Genetic Systems (9/91) and Abbott Laboratories (2/92). A memorandum to licensed blood establishments for test implementation needs to be issued in the very near future integrating recommendations from recent meetings of the Blood Products Advisory Committee. Also, an MMWR is under development to address the larger Public Health issues of testing and notification. A related problem is the lack of a licensed confirmatory test for anti-HIV-2. Through cooperation with CDC, twenty-seven State Public Health Laboratories will jointly file an IND for research use of HIV-2 Western blot tests. This is a novel approach to the problem of confirmation following newly approved screening tests and should be expedited.
2. Waldheim Pharmazeutika was recently licensed to distribute an HIV-1 IFA test (2/92). This test may be used as a confirmatory test in lieu of Western blot, or, in urgent situations, as a donor screen. A memorandum to blood establishments is in preparation to familiarize users with the FDA position on proper use of this test.
3. The first anti-HCV test based on multiple antigens was licensed very recently (Ortho Diagnostics, 3/92). This test uses a format incompatible with the equipment at most blood establishments. Abbott Laboratories is at least 6 weeks behind Ortho in development of a comparable test using a format available in 80% of establishments.

The multi-antigen HCV test will detect more infectious donors and detect them sooner than the existing test.

Consequently, FDA intends to recommend that the improved test replace the existing test as soon as feasible. For blood establishments not equipped to use the format of the approved test, there is great concern about having to change to another format and about the regulatory and liability concerns of delayed implementation.

B. Product Review

Several areas of current product review need attention from either a management or a policy perspective:

1. Resources and responsibilities need to be defined for several new areas of product review:
 - a. New review responsibility needs to be established for product submissions related to donor screening for non-viral diseases or agents, e.g. syphilis, *T. cruzi*, leishmania, borrelia (Lyme disease), *Yersinia enterocolitica*, and malaria. For example:
 - i. Consideration of the sponsor's complaints with the FDA review process related to the ADI Diagnostics Visuwell Reagin Test have led to the conclusion that CBER should directly review claims related to blood bank testing for syphilis tests independent of the CDRH review under 510(k) and independent of the CDC classification of approved tests.
 - ii. Concerns over transfusion transmission of *T. cruzi*, *Y. enterocolitica*, and leishmania have been subjects of recent national concern. CBER has sponsored workshops, conducted national surveillance studies and issued policy positions in these areas but still lacks a program infrastructure to deal either with the concerns or the technology solutions.

For now, the Laboratory of Hepatitis has been asked to assist in review of 510(k) submissions.

- b. New review responsibility needs to be established for evaluation of leukocyte filters and for development of license standards for leukocyte-poor products.
 - c. Product standards need to be established for intra-operative blood salvage. Program activity is needed to address many concerns about the equipment which is used.

- d. Product standards need to be established for irradiated red cells.
 - e. FDA needs to define requirements for computer software used to support release testing of blood components. This is especially important in the areas of infectious disease testing and equipment automation.
2. Approval of some products will require modification of regulations or the granting of exemptions:
- a. Biotest: Microplate for ABO/Rh testing. The existing provisions of 21 CFR 660 do not apply.
 - b. Diamed: "Gel" filtration system for ABO/Rh/Antibody screening. Changes to 21 CFR 660 will be necessary before the product can be approved.

C. Development of FDA Policy Positions and Regulations

A number of ongoing activities and several new initiatives have raised policy issues which will necessitate the establishment of an FDA policy and/or the promulgation of a regulation:

- 1. The Quality Assurance initiative for blood establishments will require an FDA position on the application of the 21 CFR 211 series to blood establishments. Prior to this initiative, blood centers were responsible only for compliance with the 600 series for cGMP. Inspector and reviewer training is needed to permit redesign of FDA inspection of blood establishments consistent with the initiative. Computer guidelines are needed for blood facilities.
- 2. In a number of areas of blood bank practices, state laws have come into conflict with federal regulations. The Agency must declare preemption regarding pertinent standards of practice, reagent qualifications and test requirements.
- 3. A proposed rule for "look-back" for HIV is awaiting approval at DHHS. FDA has been criticized for having failed previously to mandate this activity by blood centers. Rapid implementation is now expected by DHHS.
- 4. FDA has a restrictive policy on the approval of devices for home sample collection for HIV testing which has been contested by a product sponsor. (FDA's position is that

the sponsor must demonstrate a public health benefit of such an anonymous test system and also show the adequacy of telephone counseling.) University Hospital Laboratories' PMA for "AIDS Check" was found non-approvable in August, 1990. The company has requested a Section 12 hearing to reexamine the FDA's decision. If the FDA denies the hearing, then a court proceeding may follow, if the company chooses to contest the denial.

5. An inter-agency task force led by the FDA has concluded that FDA should regulate tissue transplantation. The proposal is being prepared as a report for Dr. Mason. The recommendation is for "minimum" regulation including registration of tissue banks, standards for donor screening and requirements for record keeping sufficient to trace donors or recipients in either direction. It has been proposed that CBER should carry out this function on the basis that it resembles regulation of blood establishments and sperm banks.

Alternatively, the National Tissue Bank Council has proposed legislation to amend the FD&C Act to provide for development of standards by the private sector leading to issuance of monographs to regulate human tissue for transplantation. CBER would be responsible for a significant portion of the regulatory activity related to development of monographs and intervention in situations of immanent health hazards. The Commissioner's staff is engaged in a dialogue with the NTBC on this matter.

6. A set of policy issues need to be developed in the area of Source Plasma collection:
 - a. Prison plasma collection should be eliminated because of hepatitis and HIV risks.
 - b. FDA should consider whether to discontinue HIV positive plasma collection programs except under IND.
 - c. FDA should reassess the impact on blood safety of its current definitions of paid vs. volunteer donations.
7. A field guidance document is under development to clarify FDA's expectations regarding call-back reporting by donors in the context of Error and Accident reporting.
8. A policy position needs to be developed on invalidation of test runs in infectious disease testing. Part of this issue involves an FDA position on appropriate use of reagent standards external to the test kits.

9. FDA needs to establish a policy on use of sterile connecting devices in different areas of component preparation in the blood bank.
10. A regulatory policy is needed for fibrin glue.
11. FDA must decide whether to pursue development of a national donor deferral registry.

D. Other Areas of Active Problem Solving

1. Tests are currently under development for screening saliva and urine for HIV antibody. Low levels of antibody raise sensitivity concerns. Accuracy in comparison to testing of blood needs to be rigorously established. Interim use of unapproved kits by the insurance industry has been interdicted. It is possible that this FDA action may be contested in court.
2. Reference Reagents and Lot Release

Establishment of serum panels for lot release is an ongoing activity. Current needs include:

- a) Development of a panel for HIV-2 due to recommendations for HIV-2 testing
- b) A replacement panel for HIV-1 with a higher sensitivity standard
- c) Development of a panel for HTLV-II
- d) Development of a reference panel for HIV PCR
- e) Development of a panel for HIV-1 antigen
- f) Replacement of several hepatitis panels

Concurrent with these activities, an effort is needed to decrease lot release testing from 100% testing to surveillance testing for retroviral test kits.

3. False positive screening test results (HIV, HTLV, HCV) have been associated with various immunizations, especially the flu vaccine. Several manufacturers' kits are involved. Biochemical basis appears to be an IgM binding non-specifically to the solid phase plastic in the kits. The problem can potentially be eliminated by changes to the sample diluent or other other kit modifications. Expedited approval of product amendments may be necessary to address the problem. Also, consideration must be given to reentry of donors who have exhibited these reactions.
4. Bacterial contamination of blood products for transfusion is an infrequent but refractory problem. Increased

efforts are underway to prevent fatalities particularly from contaminated platelets.

5. Thimerosal availability has become problematic: Elanco (Lilly) has terminated distribution. Thimerosal is used in many biological products, especially kit components. Manufacturers must be made aware of the shortage, find alternative preservatives, and have those formulations approved by FDA.
6. Use of anti-HBs test kits for Hepatitis B vaccinees has raised a new concern: Approved anti-HBs test kits have never been standardized to lower sensitivity limits nor standardized against the WHO standard. As a result, 5% of vaccinees with a positive test may not have sufficient protection. FDA has developed a draft statement for manufacturers to include in their product inserts to alert users that a positive test may not equate to protective antibody levels. CDC is developing an MMWR. A clinical protocol is being developed to estimate the number of individuals that could be affected.
7. Several FOI requests have been made for information related to circumstances surrounding the Abbott PLA Amendment to improve the sensitivity of their HIV-1 kit in 1986. An investigation by Congressman Dingell followed publication of an article in Chicago Tribune. Copies of all Retrovirology files have been provided to Mark Elengold for evaluation in response to the several

E. Issues Related to Resources and Program Infrastructure

1. DTS needs a data base that facilitates tracking and information access without reentering all information in each office - e.g., we can look at Data General files? Additionally, we need to initiate another system to enter and track data related to our part of the process.
2. ADP equipment orders take many months, and are unpredictable. DTS has been waiting for 15 systems for 9 months.
3. Effort needs to be focused to shorten the length of time required to publish regulations or FR notices.
4. The consensus process (DTS/OC/DPC) is very slow. Some mechanism for establishing priority responses is needed for daily policy making needs.
5. There is a lack of clearly defined areas of responsibility for DTS/OC/DPC. Significant overlaps

exist which need to be reconciled.

6. Personnel actions take enormous amounts of effort and time. We need someone to facilitate getting things done. Each Division faces a problem of inefficiency in this area.
7. Clerical staff ceilings (e.g., one GS-6 in DTS/Director's office) should be eliminated.
8. In the Laboratory of Blood Bank Practices, non-laboratory scientist positions are needed to recruit Ph.D. or M.D. experts to lead sections in:
 - 1) QA
 - 2) Devices/Computers
 - 3) Donor selection/protection/deferral
 - 4) Reagents
 - 5) Training/Communication

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DIVISION OF TRANSFUSION SCIENCE
LABORATORY OF BLOOD BANK PRACTICES

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March 17, 1992/DENHAM/CONTENTS

Revised June 9, 1992

Revised June 10, 1992

Revised June 30, 1992

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[DOCKET NO.91N-0450]

QUALITY ASSURANCE WORKSHOP
BACKGROUND INFORMATION

January 1992

For further information about this document, contact:

Center for Biologics Evaluation and Research (HFB-900)
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
301-227-6700

Submit written comments on this document to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
Rm. 1-23
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Food and Drug Administration
5600 Fishers Lane
Rockville, MD. 20857
301-295-8228
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QUALITY ASSURANCE IN BLOOD ESTABLISHMENTS

INTRODUCTION

The United States has one of the safest blood supplies worldwide. Yet, the Food & Drug Administration (FDA) has noted in recent years a significant increase in the number of deficiencies observed during blood bank inspections relating to the release of unsuitable blood and blood components. As a result, the Center for Biologics Evaluation and Research issued a memorandum to all registered blood establishments on March 20, 1991, listing the more significant errors and deficiencies. This memorandum was issued to assist the industry in conducting self audits.

These developments may be related to: (1) the increase in the number of tests performed on blood and blood products (increased testing increases the opportunity for errors and contributes to the complexity of the operation); (2) the use of advanced technology in testing procedures and equipment including computer systems; (3) shortage of appropriately trained health care and laboratory personnel; and (4) the need for more sophisticated control procedures in blood banks including those involving training and supervision of existing personnel.

Because blood establishments must provide and maintain a safe blood supply, it is essential that the industry develop well planned, written, and managed quality assurance programs that address the causes of recent problems in blood establishment performance. Although there is no way to guarantee 100% accuracy, the goals of quality assurance are to significantly decrease errors, lend credibility to results, improve product safety and quality, and ultimately improve productivity and reduce costs. Quality assurance includes measures to investigate, detect, assess, correct and prevent errors. The emphasis is on preventing errors rather

than detecting them after the fact.

Implementing a quality assurance program requires a commitment of time and resources. The extent of a quality assurance program depends on the size and complexity of the establishment. All establishments, regardless of size, should invest in quality assurance. The potential public health consequences demand this commitment.

QUALITY ASSURANCE SYSTEMS

There are several dimensions to quality assurance systems in common use including quality control procedures and current good manufacturing practices (CGMPs). Quality control procedures are part of quality assurance procedures and involve the routine monitoring of manufacturing procedures. The CGMPs include the requirements in the regulations [21 CFR 211.22 (a) & 211.100 (a)] and other standards of industry practice relevant to the control of production. These include standards for personnel, facilities, procedures, equipment, testing, recordkeeping, and quality control activities.

Quality assurance programs are those aspects of CGMPs that are in place to assure that manufacturing is consistently carried out in such a way as to yield a product of purported quality. Quality assurance is the sum of the activities planned and performed to provide confidence that all systems and their elements that influence the quality of the product are working as expected.

QUALITY ASSURANCE UNIT

Reporting Responsibilities:

A quality assurance unit (QA Unit) should report to top management. If the firm is licensed, the specific individual to whom the QA Unit should report is the Responsible Head. This is the individual designated in the establishment license application to represent the firm in its regulatory activities with the Food and Drug Administration as prescribed in 21 CFR 600.10 (a). If the firm is

not licensed, the individual should be a "designated qualified person" as prescribed in 21 CFR 606.20 (a).

The Responsible Head or the "designated qualified" person is required to exercise control over the establishment in all matters related to compliance with FDA requirements. This individual is the chief official or representative of top management in a blood establishment charged with the obligation to assure the quality of products manufactured in that establishment. This individual is also accountable under the regulations for assuring that personnel are appropriately assigned and trained to accomplish their duties.

A schematic of the interaction among the Responsible Head or "designated qualified person," production, and the Quality Assurance Unit is shown in Figure 1. In this diagram, the QA Unit reports independently to top management, and is separated from any responsibility or authority over production. Top management in a facility must be informed of the findings of the QA Unit and must have the authority to assure that corrective action is taken when necessary.

QUALITY ASSURANCE UNIT RESPONSIBILITIES

The responsibilities of a QA Unit in blood establishments should include, but may not be limited to, the following areas:

Standard Operating Procedures (SOPs)

Quality assurance activities relevant to SOPs include:

- * Determination that SOPs exist for all manufacturing and testing procedures, and that SOPs accurately describe and define the procedure, including a statement of what the procedure is intended to accomplish.
- * Review and approval of all (SOPs) prior to implementation, and confirmation that SOPs are in compliance with all applicable regulatory



requirements. In addition, the QA unit assures, prior to implementation of each SOP, that:

- (a) there are written procedures for validation to assure that the procedure accomplishes its intended purpose;
- (b) the individuals who will be responsible for performing the SOP have been identified;
- (c) procedures have been developed for training and certifying these individuals;
- (d) the responsibilities of supervisors for oversight of the performance of all procedures have been described;
- (e) methods for periodic proficiency testing (or evaluation) of the individuals performing the procedure are described;
- (f) methods for evaluating the performance of the procedure during quality assurance audits are described; and,
- (g) a designation is made regarding whether or not the procedure is considered a critical control point.

- * Maintenance of an index of all SOPs, a master copy, and an archive of obsolete SOPs.
- * Assurance that each employee is provided with, and has ongoing access to, the necessary SOPs to perform assigned duties.
- * Assurance that modifications or changes in SOPs are appropriately documented including rationale for the change. Assurance that the new procedure is validated to verify that changes were valid and did not create an adverse impact elsewhere in the

system. Changes in SOPs should be made in accordance with a written procedure and be formally approved and implemented.

Training and Education

The QA Unit should assist in developing, reviewing, and approving all training and educational programs for all personnel. Training programs may include:

- New employee orientation
- CGMP training
- SOP training
- Technical training
- Quality Assurance training
- Supervisory training
- Managerial training
- Continuing education and training

The QA Unit should be aware of factors that indicate a need for training or retraining. This information regarding the need for retraining may be derived from management observations, proficiency test results, technical changes, error/accident reports, complaints, quality assurance audits, and problems discovered at critical control points identified in every system within the establishment's total operation. Thresholds for implementation of retraining programs should be established.

Job Descriptions

The QA Unit should assist in preparing, reviewing, and approving all employee job descriptions including detailed descriptions of supervisory and upper management responsibilities. The QA Unit should approve the criteria for employee credentials based upon the job description.

Proficiency Testing

Proficiency tests are commonly one part of the quality control program for testing laboratories. The QA Unit should review, evaluate, and monitor the proficiency testing program to assure the adequacy of test methods and equipment, and competency of personnel performing proficiency testing. Proficiency testing programs should assure that proficiency samples are actually tested by the personnel normally performing the routine testing, using the routine test equipment, during routine test runs. Procedures should be implemented to assure accurate, reliable, and prompt test result reporting. There should be a plan for corrective action in the event that the laboratory fails to pass proficiency testing. Other quality control procedures that may be useful in monitoring laboratory performance include statistical reviews of unknown and control sample results, blinded check samples, and the comparison of initial and repeat test values.

Validation

Because of the great variety of systems and processes in place, it is not possible to state in one document all of the specific validation elements that are applicable to all blood establishments. Validation is defined as, "Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics", as referenced in FDA's Guideline on General Principles of Process Validation (May 1987).

Equipment Validation, Maintenance, and Monitoring

Equipment validation is performed to establish

"confidence that process equipment and ancillary systems are capable of consistently operating within established limits and tolerances" (Guideline on General Principles of Process Validation; May, 1987). There should be quality assurance procedures in place for equipment qualification and validation to assure the proper function of all equipment, and maintenance of appropriate records of the results of validation testing. There should be written procedures and schedules for equipment maintenance and revalidation sufficient to assure that it will perform according to specifications. There should also be programs for continuous monitoring of performance.

Validation of computer systems used in blood establishments is an important aspect of validation. FDA intends to prepare a guideline regarding blood bank computer systems in the near future.

Error/Accident Reports, Complaints, and Adverse Reactions

The quality assurance system should provide for a program to assure that procedures are in place and followed for the review, evaluation, investigation, and correction of manufacturing errors and accidents. There should also be a system to assure the reporting of all error/accidents pursuant to 21 CFR 600.14. Quality assurance procedures should be in place to assure that all complaints regarding product quality are investigated to determine whether the complaint is related to an error/accident in manufacturing. Investigative procedures should include provisions for review to determine whether the complaint represents an adverse reaction. Donor and/or recipient adverse reactions may be life-threatening, permanently disabling, or fatal.

Procedures should be in place to assure that donor and recipient adverse reactions are investigated and documented. Fatalities must be reported pursuant to 21 CFR 606.170 (b).

Procedures should be in place to assure that transfusion reactions are investigated and that possible bacterial contamination of the product is recognized. Procedures should be in place to review manufacturing procedures when bacterial contamination of the product is suspected. A program should be in place to train patient care staff to recognize symptoms of adverse reactions so that appropriate intervention can be taken.

Investigation of complaints and error/accident incidents feed back into the system as an essential element of quality assurance. If there is no investigation of complaints or errors, then factors that contributed to the problem cannot be identified and corrected. Errors and accidents in manufacturing may be identified either by employees in the course of routine activities or by supervisors during review of activities. The QA Unit must assess all errors that occur during manufacturing including those identified before products are released. There should be thresholds for initiating retraining related to errors and accidents.

Recordkeeping

The QA Unit should approve all recordkeeping systems (manual and automated). Quality assurance procedures should be implemented to assure that records required to be maintained pursuant to 21 CFR 606.160 are reviewed as necessary to assure the accurate history of all work performed.

Lot Release Procedures

Each component (e.g., red blood cells, platelets, plasma, and cryoprecipitated AHF) prepared from a unit of whole blood represents one lot of product, bearing a unique lot number which is the unit number assigned at the time of collection. Quality assurance procedures should be implemented to assure that manufacturing records for each step in each process associated with every component produced are reviewed prior to making the product available for release as prescribed in 21 CFR 606.100 (c). Quality Assurance procedures should be in place to assure that any lot discrepancies or failure of a lot or unit to meet its specifications are investigated.

Quality Assurance Audits

An internal audit is one mechanism for monitoring the effectiveness of the total quality assurance system. Audits are conducted in accordance with approved written procedures by knowledgeable personnel on a periodic basis. Increased audits may be necessary when quality problems have been identified, or to monitor more effectively a particularly critical area. (Isolated audits restricted to one area may not detect system-wide problems). The audit procedures will vary in complexity depending on the size of the establishment and the specific process under review. Individuals responsible for conducting the audits need sufficient knowledge, training and experience to identify problems in the specific process under review. The auditor or audit team should be individuals who are not responsible for performing those procedures being audited.

Audit Reports

There should be a written report documenting the audit procedures and results. There should be a plan to review

and evaluate the results of the audit by the Responsible Head or "designated qualified person" and top management. This review and evaluation is to assure that corrective action is implemented by the person with the authority to effect change.

FDA has established a policy not to routinely review certain internal audit reports that meet the criteria specified in Compliance Policy Guide 7151.02. "This policy applies to any regulated entity which has a written quality assurance program that provides for periodic audits or inspections. FDA may seek written certification that such audits and inspections have been implemented, performed, and documented and that any required corrective action has been taken. In addition, FDA may seek access to reports and records of such audits and inspection during a "directed" or "for cause" inspection of a sponsor or monitor of a clinical investigation, during litigation (under applicable procedural rules), or by an inspection warrant where access to records is authorized by statute. FDA will continue to review and copy records related to quality control investigations of product failures and manufacturing errors."

DESIGN OF AUDITS

Quality assurance audits should be structured using a systems approach. Each major manufacturing operation is identified as a system.

- * Quality Assurance
- * Donor Suitability
- * Blood Collection
- * Component Manufacturing
- * Product Testing
- * Storage and Distribution

- * Lot Release
- * Computerization

Each system functions independently and collectively. The critical control points in each system are steps that if not performed or functioning correctly may affect the safety and quality of the product.

Quality assurance audits should evaluate the critical control points in each system. Examples of critical control points and associated key elements are included in the attached tables.

APPENDICES

FIGURE 1

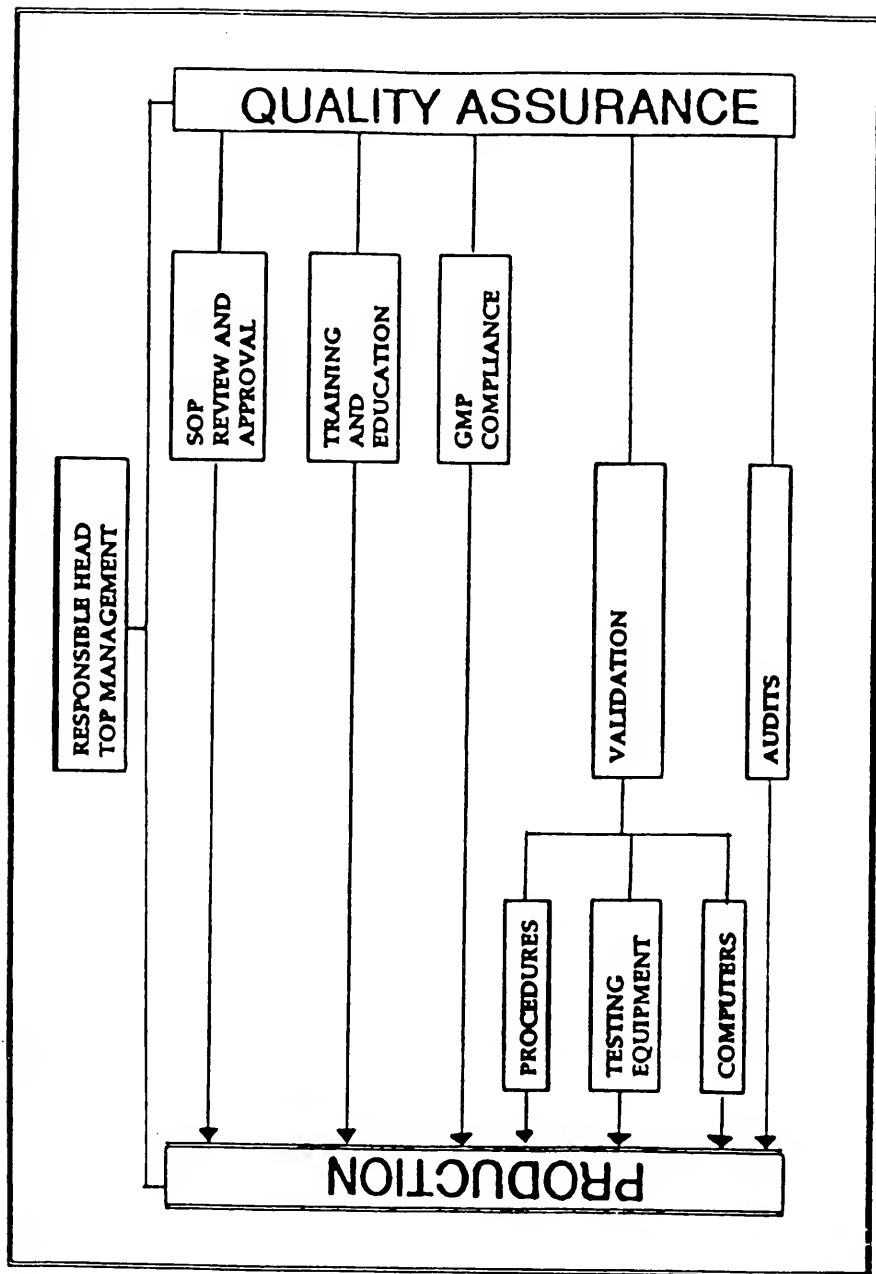


FIGURE 1

TABLES OF SYSTEMS

Critical Control Point:

QA Unit Established Separate from Production

Key Elements:

Objectives/Policies
Product Specifications/Validation
Standard Operating Procedures/Validation
Training in Assigned Duties, QA and CGMP for Every Employee/Competency
CGMP/Regulatory Compliance
Industry Standards

Critical Control Point:

Quality Control

Key Elements:

Product Testing
Equipment Testing
Reagent Testing

Critical Control Point:

Audits

Key Elements:

Responsible Personnel
Systems Working Separately and Collectively
Thresholds (Limits)
Alert Levels/Action Levels
Written Audit Reports
Report Evaluation/Data Analysis
Feedback
Corrective Action

Critical Control Point:

Equipment Maintenance/Repair

Key Elements:

Preventative Maintenance
Routine/Scheduled Maintenance
Maintenance Records
Qualification/Validation After Repair
Repair Records

S Y S T E M : D O N O R S U I T A B I L I T Y

**Critical Control Point:
Key Elements:**

Donor Registration
Accurate Information Provided by the donor
Accurate Information Entered in record files
Certainty of Identifiers/Permanently Effective
Method to Prevent Known Unsuitable Donors from Donating (Example: Use
of Donor Deferral Registry at the time of registration)
Record Review

**Critical Control Point:
Key Elements:**

Donor Screening
Privacy/Effective Communication
Consistent Application of all Health History and Screening Criteria
Equipment/Reagents
Informed Consent
Quality Control/Record Review
AIDS Education Information/Questions on High Risk Behavior
Donor Medical History/Donor Medical Examination
Confidential Unit Exclusion (C.U.E.)

**Critical Control Point:
Key Elements:**

Donor Acceptance
Acceptance Criteria
Documentation by Authorized Personnel/Supervisory Concurrence

**Critical Control Point:
Key Elements:**

Donor Deferral Registry
Accurate Information/Positive Identification
Availability at Donation Site
Resolving Discrepancies/Duplicate Information
Adding/Deleting/Changing Information
Transferring Information
Access to Information/Data Retrieval
Permanent vs. Temporary Conditions/Deferral Time
Record Review

**Critical Control Point:
Key Elements:**

Donor Deferral
Notification-Donor/Health Authorities
Additional Testing
Counseling/Education
Re-entry Algorithm

S Y S T E M : B L O O D C O L L E C T I O N

Critical Control Point:

Blood/Component, Source Plasma Collection

Key Elements:

Identification of donor, containers, pilot samples, components, records
Verification that donor corresponds to container, pilot sample,
components, records
Arm Preparation
Equipment/Supplies/IV Solutions
Collection Set/Container Type/Anti-Coagulant
Donor Adverse Reactions
Overbleeding/Underbleeding/Product Meeting Specifications
Automated Collection/Multiple Component Harvesting
Volume of Product Retained/Returned to Donor
Material Defects/Procedural Errors
Record Review

Critical Control Point:

Donor Immunization Program - Source Plasma

Key Elements:

Antigen Safety, Purity and Potency/Specifications
Antigen Selection and Administration/Schedules and Doses
Donor Antibody Production and Evaluation
Product Specifications for Labeled Use/Discard
Disposition of low titer or other unsuitable product
Donor Medical Record Review/ Adverse Reactions
Product Record Review

Critical Control Point:

Component Preparation - Products Intended for Transfusion

Key Elements:

Preparation Time/Temperature/Equipment
Assignment of Expiration Date
Anticoagulant/Additive Solution/Container
Sterile Connecting Device/Pooling Apparatus/Other Devices
Record Review/Quality Control
Disposition of All Products

Critical Control Point:

Component Preparation - Further Manufacturing

Key Elements:

Preparation Time/Temperature/Equipment
Storage Time/Temperature
Pooling
Expiration Date
Record Review

Critical Control Point:

Labeling

Key Elements:

Verification of Correct Labeling for Final Intended Use
Bar Coding/Eye Readable Agreement
On-line Printing Devices
On Demand/Batch Printing
Label Control
Records of Labeling Operations

Critical Control Point:

Quarantine

Key Elements:

Location of Product- Untested, Repeat Test Pending,
Biohazardous, Not Meeting Specifications, Unsuitable
for Transfusion, Autologous, Directed
Processing Record Review
Release for Distribution
Correct Labeling
Returned Products
Destruction or Other Disposition/Records
Equipment

Critical Control Point:

Sample

Key Elements:

Identification/Collection/Labeling
Detection of Missing Samples/Sample Order
Manufacturer's Instructions/Serum or Plasma
Storage Time and Temperature
Records/Sample Acquisition/Retention

Critical Control Point:

Reagents

Key Elements:

Receipt Records
Storage Conditions and Records
Manufacturer's Instructions for Use
Quality Control Testing/Daily/Acceptance of New Stock
Records of Error of Problems/Reports to Authorities

Critical Control Point:

Equipment

Key Elements:

Intended Use/Design
Interface Compatibility (if appropriate)
Qualification and Calibration
Maintenance and Repair
Employee Training/Introduction to New Equipment
Validation/Parallel Testing
Quality Control
Records

Critical Control Point:

Laboratory Testing

Key Elements:

Sample Identification/Order/Completeness
Controls/Data Calculation/Interpretation
Reactive/Positive Test Results/Confirmatory Testing
Invalid Tests/Problems
Repeat Tests/Test Sequence
Test Interruption/Start Up/Shut Down/Alternative Methods
Detection of Missing Test Results
Records

S Y S T E M : L O T R E L E A S E

Critical Control Point:

Verification of Purported Labeling Claims

Key Elements:

Record Review: All Testing, required and recommended, was performed according to manufacturer's instructions and SOP and the results are acceptable for release

Record Review: All Quality Control was performed and results were within acceptable limits

Record Review: All Manufacturing Records verified and approved by designated, responsible personnel (supervisor) prior to the release of every component

Record Review: Correct Expiration Date Assigned

Documentation of All Reviews

Critical Control Point:

Donor Suitability

Key Elements:

Record Review
Deferral Registry

S Y S T E M : S T O R A G E A N D D I S T R I B U T I O N

Critical Control Point:

Storage of Products

Key Elements:

Product Location/Place/Order
Products for Further Manufacturing
Products Quarantined- Untested, Repeat Test Pending,
Biohazardous, Not Meeting Specifications, Unsuitable
for Transfusion, Autologous, Directed
Products Available for Distribution
Products Available for Compatibility Testing
Return of Product
Equipment
Temperature Monitoring
Alarm System
Record Review

Critical Control Point:

Distribution

Key Elements:

Lot Record Review/Release Specifications
Expiration Date
Visual Inspection
Use of Circular
Packing/Destination
Shipping Temperature
Documentation of Appropriate Shipping Temperature
Records

SYSTEM : COMPUTER

Critical Control Point:	Documentation
Key Elements:	Specifications Hardware - listing of components, installation date, serial numbers, maintenance records Software - listing of programs, program descriptions, program interaction, development, changes, archives Validation for Systems Personnel/SOPs
Critical Control Point:	Testing the Computer System
Key Elements:	Develop and Maintain a test database/test scenarios Develop and Maintain test case protocols to cover normal, exceptional, boundary, stress, and invalid test cases Perform testing and Document results Define and Document evaluation of the test process Document corrections performed as a result of testing Equipment Interface
Critical Control Point:	Implementation
Key Elements:	Define and Document implementation process Parallel Testing Change Control Approval Process
Critical Control Point:	Maintenance
Key Elements:	Hardware Software for Applications and Operating Systems Change Control Approval Process Archives

GLOSSARY

GLOSSARY

Action Levels: A limit determined by the QA unit that indicates the need to immediately identify the source of an error and make the necessary corrections to avoid a compromise in product quality.

Alert Level: A limit determined by the QA unit that indicates that a problem needs to be identified and corrected but will not adversely effect product quality.

Critical Control Point: A step or function in a manufacturing process the correct performance of which is necessary to assure the quality of the finished product.

Current Good Manufacturing Practice (CGMP): CGMP is that part of quality assurance which ensures that products are consistently manufactured to, and controlled by, the quality standards appropriate to their intended use. It encompasses both manufacturing and quality control procedures.

Maintenance: Activities such as adjusting, cleaning, modifying, overhauling equipment to ensure performance in accordance with quality requirements. Maintenance to a software system includes correcting software errors, adapting software to a new environment, or making enhancements to software.

Parallel Testing: Functional testing performed using two or more different systems simultaneously.

Quality: Conformance of a product or process with pre-established specifications or standards.

Quality Assurance Audit: A documented independent inspection and review of a QA system, performed in accordance with written procedures on a periodic basis.

Quality Assurance: The actions, planned and taken, to provide confidence that all systems and elements that influence the quality of the product are working as expected. [All systems are working individually and collectively].

Quality Assurance (QA) Program: An organization's total system for manufacturing safe, effective, quality products according to regulatory standard. This program includes preventing, detecting and correcting deficiencies that compromise product quality.

Quality Control: A component of QA programs that includes the activities and controls used to determine the accuracy of the establishments' personnel, equipment and operations in the manufacturing of blood products.

Specification: Physical characteristics and composition,

performance characteristics, parameters, requirements, standards, intended functions, behavior or other characteristics of a system or product.

Test Cases: (as applied to computer systems)

- **Normal:** valid data sets are used to produce normal outputs;
- **Exceptional:** valid data provides an unusual twist for the program. The purpose is to force the program to react to something that might be unexpected;
- **Boundary:** force the program to evaluate conditions that are of borderline validity;
- **Stress:** determine whether the system has reached its desired level of performance
- **Invalid:** data that is not valid; test data should be designed to force a program to prove that it can detect invalid input. Examples of invalid test cases may include an invalid donation date, i.e., 02/30/91; an invalid ABO group, i.e., "P"; a negative hemoglobin value; or no data entry.

Thresholds: Guidelines or standards which are needed in reviewing records to determine the adequacy of performance. These are the acceptable limits for quality to determine when intervention is necessary. Thresholds may be expressed as a number or percentage.

Validation: Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Process validation is performed to evaluate the performance of a system with regard to its effectiveness based on intended use.

Validation Protocol: A descriptive document of a plan stating how validation will be conducted. The protocol should contain a physical description of software hardware and integration of systems; system characteristics, test parameters, and discussion points on what constitutes acceptable test results.



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